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THE THERMOGENIC EFFECT AND METABOLIC FATE OF ETIOCHOLANOLONE IN MAN*

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ABSTRACT

The intramuscular administration in man of etiocholanolone (3α-hydroxyetiocholane-17-one), a metabolite of testicular and adrenocortical hormones, consistently provoked pyrogenic and inflammatory reactions. The thermogenic response following single injections of small amounts of this steroid was characterized by the development of high fever over a period of four to twelve hours, with equally rapid defervescence. Leukocytosis, local inflammatory reactions and a variety of transient constitutional symptoms were observed as well. The reaction is structurally specific and the pyrogenic response appears to be a direct effect of the molecule itself, since metabolic studies revealed that the steroid was excreted in large part unaltered and recovery of administered compound was high. The epimeric steroid, 3β-etiocholanolone was inert with respect to production of fever. The metabolic pattern was in contrast with that of etiocholanolone, in that there was significant transformation of the 3β to the 3α-hydroxy derivative, little unchanged steroid was excreted, and overall recovery of administered compound was low.

THIS is a report of studies on the metabolism and thermogenic effect of etiocholanolone (3α-hydroxyetiocholane-17-one) in man. This substance is one of the principal urinary ketosteroids and represents a transformation product of several hormones of testicular and adrenocortical

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origin. Information on its physiologic disposition is of particular interest because of the striking pyrogenicity of this hormonal end-product in man (1, 2). The association of this activity with a high degree of structural specificity, moreover, lends interest to a comparison of its metabolism with that of the epimer 3β-hydroxyetiocholane-17-one, which does not provoke fever. The metabolic fate of both deuterium-labeled and unlabeled steroids is reported.

METHODS AND RESULTS[1]

Metabolism of 3α-Hydroxyetiocholane-17-one

The metabolism of 3α-hydroxyetiocholane-17-one (etiocholanolone) was studied in 6 men. Steroids were administered intramuscularly in sesame oil containing a small amount of benzyl alcohol. Rigid precautions were taken to exclude pyrogens. The injection vehicle was devoid of pyrogenic activity (2).

Subject B was a 26-year-old normal man. Three collections of urine from this subject were studied: Period 1, an eight-day control; Period 2, a one-day collection during which he received 200 mg. of etiocholanolone intramuscularly; and Period 3, a one-day control immediately following Period 2. The conjugates were hydrolyzed according to Method E (3), *i.e.*, incubation with β-glucuronidase[2] at pH 5 for five days followed by continuous ether extraction for forty-eight hours at pH 1. Ketonic, α-ketonic and β-ketonic fractions were prepared as described previously (4).

Subject V was a 25-year-old normal man. Three collections from this subject were studied: Period 1, a five-day control; Period 2, a one-day collection during which he received 100 mg. of etiocholanolone intramuscularly; and Period 3, a one-day post-injection control. Urines were processed as with Subject B.

Subject M was a 42-year-old man with inactive, chronic rheumatoid arthritis. Four collections from this subject were studied: Period 1, a three-day control; Periods 2 and 3, representing single-day collections during which he received 25 mg. of etiocholanolone intramuscularly per day; and Period 4, a one-day post-injection control. Urines were processed as described, and the α-ketosteroids were isolated by paper chromatography (3).

Subject G was a 40-year-old normal man. Four collections from this subject were studied: Period 1, a three-day control; Period 2, representing the second day of administration of etiocholanolone, 25 mg. per day intramuscularly; Period 3, a one-day post-injection control; and Period 4, a one-day collection on the third day after Period 2. Urines were analyzed for individual α-ketosteroids as in Subject M.

[1] The symbol AD signifies atom per cent excess deuterium and "mg.-KS" signifies milligram equivalents of dehydroisoandrosterone by a modified Zimmermann color determination. The terms "α-ketonic fraction" and "α-ketosteroids" are used conventionally to indicate substances not precipitable by digitonin under the prescribed conditions (4). Similarly "β-ketonic fraction" and "β-ketosteroids" signify compounds that form insoluble digitonides. All melting points are corrected and were determined on a micro hot-stage.

[2] β-Glucuronidase, known as Ketodase, was obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Company, New York, N. Y.

Subject S was a 30-year-old normal man who received a single dose of 9.0 mg. of 11,12-d_2-3α-hydroxyetiocholane-17-one (5.50 AD).[3]

Subject F was a 30-year-old normal man who received a single dose of 94.3 mg. of 11,12-d_2-3α-hydroxyetiocholane-17-one (5.50 AD).[3] Analyses of urinary steroids in Subjects S and F are described subsequently.

Clinical effects

All subjects reacted with high fevers and showed evidence of local inflammation. The course of the febrile response noted in Subject M was

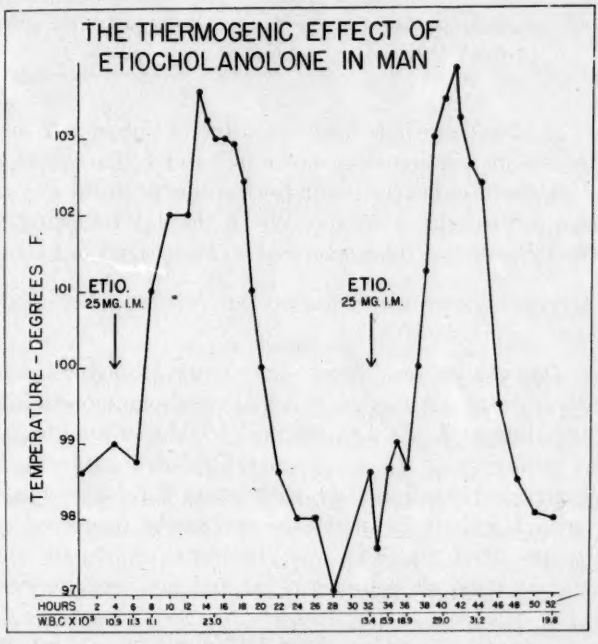

FIG. 1. The abbreviation "Etio" is for 3α-hydroxyetiocholane-17-one.

typical, and this is shown in detail in Figure 1. Within four hours of the injection of 25 mg. of etiocholanolone, body temperature increased sharply. A peak of 103.6° F. was reached within nine hours, and a rapid defervescence followed. An equally abrupt rise and fall in body temperature followed the second injection of etiocholanolone. Concurrent leukocytosis was observed.

[3] The deuterium-labeled compound was prepared by Dr. T. H. Kritchevsky from 11,12-d_2-3α-acetoxy-17α-hydroxypregnane-20-one by chromic-acid oxidation and saponification. The 11, 12-d_2-3α-hydroxyetiocholane-17-one had a melting point of 150–151°; $[\alpha]_D$+109° (ethanol); 5.50 AD.

TABLE 1. METABOLISM OF 3α-HYDROXYETIOCHOLANE-17-ONE

Recovery of steroids following intramuscular administration of etiocholanolone—100 mg. in Subject V and 200 mg. in Subject B. Both injections were during Period 2

Subject	Fraction	Milligrams per 24 hours		
		Period 1	Period 2	Period 3
V	α-Ketonic KS	13.6	98.1	21.1
	β-Ketonic KS	7.0	15.4	6.8
B	α-Ketonic KS	15.4	153.0	30.2
	β-Ketonic KS	6.2	13.3	5.9

Recovery of 17-ketosteroids from the urine of Subjects V and B following injection of etiocholanolone is shown in Table 1. There was a very large increase in α-ketosteroid excretion in both subjects on the day of injection, with a return to virtually normal levels on the day following. A small increase in the excretion of β-ketosteroids was observed in both men.

Isolation of urinary steroids following administration of 3α-hydroxyetiocholane-17-one (etiocholanolone)

Subject S. Day of injection: The 24-hour urine following the intramuscular administration of 9.0 mg. of 11,12-d_2-etiocholanolone (5.50 AD) was processed by Method A (5), *i.e.*, refluxed for thirty minutes after acidification with sulfuric acid to a concentration of 5 per cent (V/V). The urine was extracted continuously with ether for forty-eight hours and separated into fractions by methods previously described (4). The α-ketonic fraction (29.7 mg.-KS) was chromatographed on alumina. The results of the isolation of isotopically labeled metabolites are recorded in Table 2.

The eluates containing etiocholane-3,17-dione were diluted with 20.89 mg. of carrier etiocholanedione. Purification by sublimation and recrystallizations yielded etiocholanedione, m.p. 135–136°; 0.031 AD. This corresponds to 0.11 mg. of administered steroid excreted as etiocholane-3,17-dione, based on calculation (6); endogenous production was disregarded.

The eluates containing 3α-hydroxyandrostane-17-one (androsterone) were diluted with 20.83 mg. of carrier androsterone. After purification, androsterone had a normal abundance of isotope.

The eluates containing etiocholanolone were sublimed to yield 13 mg. of steroid. Recrystallizations from ether-petroleum ether and benzene-petroleum ether yielded etiocholanolone, m.p. 134–140° transition and then

TABLE 2. EXCRETION OF METABOLITES FOLLOWING INTRAMUSCULAR INJECTION OF 11,12-d_2-3α-HYDROXYETIOCHOLANE-17-ONE

Steroid isolated	Milligrams per 24 hours	
	Subject F (dose, 94.3 mg. i.m.)	Subject S (dose, 9.0 mg. i.m.)
Day of Injection		
Etiocholanolone	54.9	7.38
Etiocholane-3,17-dione	0.06	0.11
Etiocholane-3α,17β-diol	0.35	
Androsterone	0.0	0.0
Androstane-3α,17β-diol	0.0	
Total	*55.31*	*7.49*
Day after Injection		
Etiocholanolone	2.54	

146–148°; 3.06 AD. Calculations based on a total excretion of 13 mg. (the weight of the sublimate) showed that 7.3 mg. of the administered etiocholanolone was recovered unchanged, and that there was 5.7 mg. of endogenous metabolite (6). There was an additional 1.36 mg.-KS of impure etiocholanolone in the eluates preceding the foregoing fractions. Addition of 21.4 mg. of carrier etiocholanolone to this fraction, followed by sublimation and recrystallization from benzene-petroleum ether, yielded etiocholanolone, m.p. 151.5–153.5°; 0.021 AD, corresponding to 0.08 mg. of administered steroid. There was therefore a total recovery of 7.38 mg. of etiocholanolone and 0.11 mg. of etiocholane-3, 17-dione from administered steroid.

Subject F. Day of injection: The 24-hour urine collection following the intramuscular administration of 94.3 mg. of 11,12-d_2-etiocholanolone (5.50 AD) was processed as described. The α-ketonic fraction (71.4 mg.-KS) was chromatographed on alumina. The results are recorded in Table 2.

The eluates containing etiocholanedione were diluted with 25.41 mg. of carrier etiocholanedione. Sublimation and recrystallizations afforded etiocholanedione, m.p. 135–137°; 0.015 AD, corresponding to 0.06 mg. of etiocholanedione derived from administered steroid.

The eluates containing etiocholanolone (64 mg., weight; 69.6 mg.-KS) were divided into 4 equal portions. One portion was recrystallized twice from benzene-petroleum ether to yield etiocholanolone, m.p. transformation at 139°, 149–151°; 4.72 AD. To another portion (16 mg.) was added

32.0 mg. of carrier etiocholanolone. Sublimation and recrystallization from benzene-petroleum ether yielded the steroid, m.p. 140–144°, 151–153°; 1.58 AD, corresponding to 16.0 mg. of etiocholanolone with 4.72 AD. It was calculated that there was 54.9 mg. of etiocholanolone derived from administered steroid and 9.1 mg. derived from endogenous sources in the chromatographic eluates.

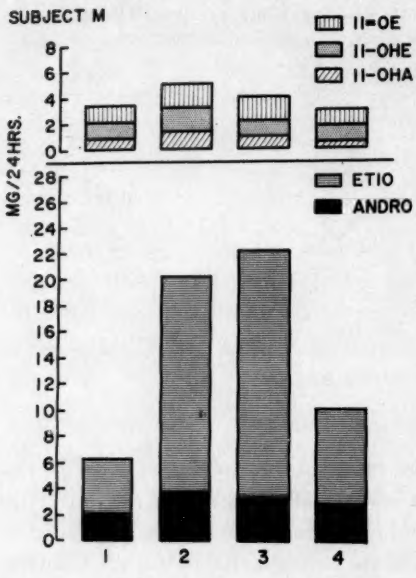

FIG. 2. Recovery of steroids following intramuscular administration of 25 mg. of etiocholanolone during Periods 2 and 3. (Period 1, three-day control; Periods 2 and 3, etiocholanolone 25 mg. per day; Period 4, one-day control immediately following Period 3. The height of the bar indicates the sum of the individual steroids represented.) (Abbreviations: 11-OE for 3α-hydroxyetiocholane-11, 17-dione; 11-OHE for 3α, 11β-dihydroxyetiocholane-17-one; 11-OHA for 3α, 11β-dihydroxyandrostane-17-one; Etio for 3α-hydroxyetiocholane-17-one; Andro for 3α-hydroxyandrostane-17-one.)

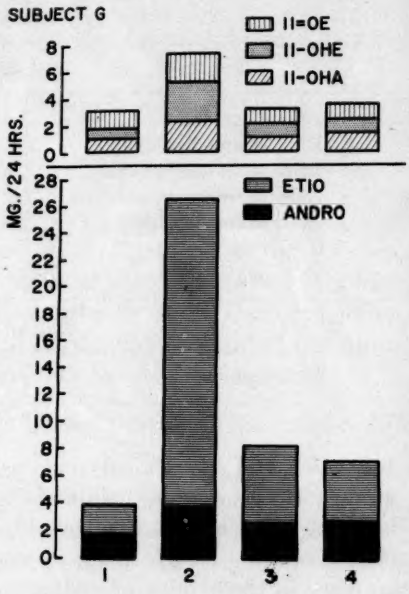

FIG. 3. Recovery of steroids following intramuscular administration of 25 mg. of etiocholanolone during Period 2 and on the day preceding Period 2. (Period 1, three-day control; Period 2, second period during which the patient received 25 mg. of etiocholanolone daily; Period 3, one-day post-injection, control; Period 4, one-day control on the third day after Period 2. The height of the bar indicates the sum of the individual steroids represented.) (Abbreviations as in Figure 2.)

The non-ketonic fraction (69 mg.) was acetylated and chromatographed on alumina. The eluates containing etiocholane-3α,17β-diol diacetate were

diluted with 30.6 mg. of carrier steroid. Saponification and recrystallization yielded etiocholanediol, m.p. 235°; 0.075 AD, corresponding to 0.35 mg. of administered steroid. Endogenous production was disregarded

The eluates of the non-ketonic fraction which contained androstane-3α,17β-diol diacetate were diluted with 20.0 mg. of carrier steroid. Purification and analysis showed that androstanediol had a normal abundance of deuterium.

Subject F. Day after injection: The urine of Subject F for the following twenty-four hours was processed as described. The α-ketonic fraction was diluted with 9.6 mg. of carrier etiocholanolone and chromatographed on alumina. Purification of the eluted steroid by sublimation and recrystallization yielded etiocholanolone, m.p. 140–142°, 150–151°; 1.15 AD, corresponding to 2.54 mg. of administered steroid. There was therefore a total recovery of 57.44 mg. of etiocholanolone, 0.06 mg. of etiocholane-3,17-dione and 0.35 mg. of etiocholane-3α,17β-diol from the administered etiocholanolone.

Subjects M and G: Recoveries of isolated steroids in these subjects are shown in Figures 2 and 3. In Subject M (Fig. 2) the excretion of etiocholanolone increased sharply on the day of injection, remained elevated during the second injection day and then decreased to virtually normal levels. A significant increase in 11-oxygenated 17-ketosteroids was observed—a reflection presumably of the adrenal response to the associated febrile episode The small increase in androsterone may be accounted for similarly. In Subject G (Fig. 3) steroid excretion was studied only during the second day of injection. The increase noted in α-ketosteroids consisted principally of etiocholanolone. As in Subject M, a small increase in androsterone was observed. The 11-oxygenated steroids rose to levels more than twice those found in the control period. Steroid excretion returned to essentially control levels in both subjects shortly after the last injection of etiocholanolone.

Metabolism of 3β-Hydroxyetiocholane-17-one

The metabolism of 3β-hydroxyetiocholane-17-one (3β-etiocholanolone) was studied in 2 men. The steroid was administered intramuscularly in sesame oil-benzyl alcohol, as for the 3α-hydroxy epimer.

Subject P was a 25-year-old normal man who received 100 mg. of 5,6-d_2-3β-hydroxyetiocholane-17-one (7.70 AD).[4] A single 24-hour collection of urine was studied.

[4] The deuterium-labeled compound was obtained from the platinum-catalyzed reduction with deuterium gas of dehydroisoandrosterone acetate in acetic acid-d (CH_3-COOD). The reduction product was oxidized with chromic acid, saponified and chromatographed on alumina. The analytical sample of 5,6-d_2-3β-hydroxyetiocholane-17-one used in the metabolic study melted at 154.5–156°; $[\alpha]_D$+93.8° (chloroform); 7.70 AD.

TABLE 3. EXCRETION OF METABOLITES FOLLOWING THE INTRAMUSCULAR ADMINISTRATION OF 100 MG. OF 5,6-d_2-3β-HYDROXYETIOCHOLANE-17-ONE TO SUBJECT P

Steroid isolated	Milligrams per 24 hours
Day of Injection	
Etiocholanolone	12.9
3β-Etiocholanolone	0.6
Etiocholane-3,17-dione	0.26
Total	*14.76*

Subject W was a 27-year-old normal man who received 100 mg. of 3β-etiocholanolone Three urine collections (including pre- and post-injection controls) were studied.

No adverse reactions were noted following the injection of this steroid in either patient.

Isolation of urinary steroids following administration of 3β-hydroxyetiocholane-17-one (3β-etiocholanolone)

Subject P. Day of injection: The 24-hour urine collection following intramuscular administration of 100 mg. of 5,6-d_2-3β-hydroxyetiocholane-17-one (7.70 AD) was processed as described for Subjects F and S. The α-ketonic fraction (34.8 mg.-KS) was chromatographed on alumina. The results are recorded in Table 3.

The eluates containing etiocholane-3,17-dione were diluted with 24.42 mg. of carrier steroid. Purification by sublimation and recrystallization afforded etiocholanedione, m.p. 134–136°; 0.087 AD, corresponding to 0.26 mg. of administered steroid.

The eluates containing 3α-hydroxyetiocholane-17-one were sublimed to yield 17 mg. of this metabolite. Recrystallization yielded etiocholanolone, m.p. 140–142° transition, then 149.5–150.5°; 6.31 AD. Based on 17 mg. of sublimate, there was 13.9 mg. of etiocholanolone derived from administered 3β-etiocholanolone and 3.1 mg. from endogenous sources. The mother liquors and the chromatographic eluates preceding the fractions containing etiocholanolone were combined. Carrier 3β-etiocholanolone (28.68 mg.) was added and the mixture treated with digitonin. Recrystallization of the digitonin-precipitable fraction yielded 3β-etiocholanolone, m.p. 154–156°; 0.159 AD, corresponding to 0.60 mg. of administered steroid. The β-ketonic fraction obtained from the neutral extract contained only a small amount (0.1 mg.-KS) of ketonic material and therefore was not investigated. A total of 13.9 mg. of etiocholanolone, 0.60 mg. of 3β-

etiocholanolone and 0.26 mg. of etiocholane-3,17-dione was recovered on the day of injection from the administered 3β-etiocholanolone.

Subject W (Fig. 4) was a 27-year-old normal man. Three urine collections were studied: Period 1, a three-day control; Period 2, one day during which he received a single dose of 100 mg. of 3β-etiocholanolone intramuscularly; and Period 3, a one-day post-injection control. The urines were processed in the manner described for Subjects M and G. The conversion of significant amounts of 3β-etiocholanolone to 3α-etiocholanolone was again demonstrated. The increase in androsterone in Periods 2 and 3 represents an artifact of technique due to the appearance of small amounts of 3β-etiocholanolone in the α-ketosteroid fraction after treatment with digitonin (5). Since its chromatographic behavior resembles that of androsterone, its presence may result in falsely high values for the latter compound. Infrared spectrophotometry confirmed the presence of 3β-etiocholanolone in the androsterone fractions for Periods 2 and 3. A small amount of unchanged 3β-etiocholanolone was excreted by this subject during Periods 2 and 3, in addition to that contaminating the androsterone fractions.

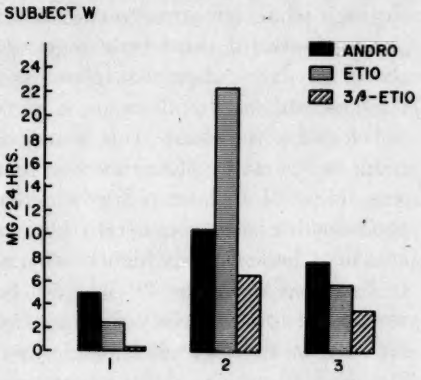

FIG. 4. Recovery of steroids following intramuscular administration of 100 mg. of 3β-etiocholanolone during Period 2. (Period 1, three-day control; Period 2, 3β-etiocholanolone 100 mg. per day; Period 3, one-day control immediately following Period 2.) The increase in androsterone is due to contamination by 3β-etiocholanolone, from which it cannot be separated by paper chromatography of the α-ketonic fraction. (Abbreviations as in Figure 2.)

DISCUSSION

The thermogenic effect of etiocholanolone in man is a striking property of a steroid previously thought to be physiologically inert. The production of fever by this metabolite of endogenous steroid hormones is associated with a strict structural specificity in the related 17-ketosteroids. Of the 4 possible compounds isomeric at C-3 and C-5, only etiocholanolone (3α, 5β) was pyrogenic. Further, as evidenced by the results of *in vivo* metabolism experiments, this pyrogenic activity of etiocholanolone is an inherent property of the molecule and is not achieved by significant transformation to another compound. This structural specificity extends to certain C_{21} ster-

oids as well, since the related compound pregnanolone (3α-hydroxypregnane-20-one), a metabolite of progesterone, has been shown to be pyrogenic (2), whereas allopregnanolone (3β-hydroxyallopregnane-20-one), also a progesterone end-product, is inert in this respect. It is possible that the slight thermogenic capacity of progesterone (7, 8) may be mediated through transformation to metabolites of the type described.

The course of the febrile reactions in Subject M (Fig. 1) is typical of those regularly observed following the intramuscular administration of etiocholanolone. In all cases, a significant increase in body temperature developed within four to six hours after the injection of the steroid. Maximum temperature elevation was reached within nine to twelve hours and was followed by a rapid subsidence of fever. The febrile response was reproducible, and concurrent leukocytosis was frequently observed. The transient leukopenia which has been observed during the febrile reactions to bacterial pyrogens (9) was not found in steroid-induced fevers. There were local inflammatory reactions of variable intensity at the sites of injection. In Subjects M and G, however, local evidence of inflammation was limited to the development of tenderness and moderate aching on movement or on pressure over the site of the injection. In both these subjects adrenocortical secretion of 11-oxygenated steroids was enhanced during the febrile episodes (Figs. 2 and 3). The elevations in excretion of β-ketosteroids observed in Subjects B and V (Table 1) can probably be attributed to the formation of small amounts of 3β-hydroxyetiocholane-17-one. Temporary elevations in pulse rate and systolic blood pressure were noted in Subjects M and G, as expected. In addition, both complained of mild anorexia and transient migratory myalgias on the days of injection. In most subjects there were various additional constitutional symptoms, including headache of variable intensity, shaking chills, a sense of agitation and tremulousness, nausea, malaise, fatigue and diffuse muscular aches and arthralgias. These phenomena are probably comparable in part to certain of the reactions described by Zondek and Bromberg (10) and by Heckel (11) in their interesting studies of "endogenous steroid allergy." The signs and symptoms which developed after administration of etiocholanolone were transient in all subjects.

The continued daily administration of etiocholanolone in small doses (approximately 10 mg. per day) produced a continuing fever, which subsided shortly after the injections were discontinued. Tolerance to the intramuscular injection of this material was not observed. Pre-treatment with large doses (300 mg. per day) of cortisone for five days diminished significantly the febrile reaction to 25 mg. of etiocholanolone. The same dose of cortisone given on the day of injection alone, however, failed to influence the hyperpyrexia. Aspirin, given simultaneously with the injec-

tions of etiocholanolone, significantly inhibited the febrile response and the local tenderness. Injection of etiocholanolone in patients with hypertension, rheumatoid arthritis, rheumatic fever, or the nephrotic syndrome produced no apparent effect on the disease and the reactions observed were the same as those seen in a normal subject.

The capacity to incite significant tissue inflammation, with hyperthermia developing subsequently through mechanisms previously described in the literature (12–17), may play an important role in the pathogenesis of the fever produced by etiocholanolone. A central effect of this metabolite acting directly or through the medium of a tissue product-steroid complex should also be seriously entertained. Certain observations are pertinent to this possibility. In some subjects, small but thermogenic doses of etiocholanolone produced a minimal tissue reaction and there appeared to be a disparity between the hyperpyrexia and the degree of local inflammation. In others, objective and subjective evidence of local inflammation persisted unabated, well beyond the period of defervescence. Moreover, on the basis of the metabolic experiments, there appears to be a close association in time between the development and subsidence of fever and the absorption and disposal of the injected steroid. Finally, the possibility of a direct influence of the compound itself on the central nervous system gains support from the demonstrated capacity of related steroids to affect cerebral activity (18–20).

The metabolism of etiocholanolone is interesting in several respects. Recovery of injected steroid was unusually high in all subjects. By reverse isotopic dilution, 7.4 mg. of etiocholanolone was obtained from the 9.0 mg. administered to Subject S. Similarly 57.4 mg. was recovered from the 94.3 mg. given to Subject F. Large yields of administered etiocholanolone were demonstrated for Subjects M and G by conventional isolation of individual urinary steroids. Based on the increment of α-ketosteroid excretion, recoveries of 75–90 per cent of administered etiocholanolone were effected in Subjects B and V. These amounts greatly exceed those reported for a number of related steroids in comparable experiments (21). The recovery is particularly impressive, since these studies were based on analysis of selected subfractions only or a minimum of selected transformation products in the isotopic dilution experiments. It is evident that the technique employed in the isolation and analysis of this substance was excellent and that the cumulative efficiency of the physiologic processes concerned with the disposal of etiocholanolone was high.

A particularly interesting aspect of the metabolism of etiocholanolone is the recovery from urine of the major portion of the injected compound apparently unaltered by any process other than conjugation. In the isotopic experiments only trace amounts of etiocholanolone were metabolized

either to the corresponding diketone or the dihydroxy derivatives; as anticipated, there was no conversion to the epimer at C-5 (androsterone). It is evident from the β-ketosteroid values in these subjects that no significant conversion to 3β-hydroxysteroids occurred. The fact that etiocholanolone can exert a significant biologic action and yet be eliminated nearly quantitatively in unaltered form may be pertinent to the fundamental problem of the mechanism of hormonal action. It would appear that no structural alteration was necessary prior to, or coincident with, the production of the physiologic effect (fever) described. In this sense the termination of the biologic action of the steroid is the conjugation mechanism through which its ultimate disposal takes place. These same considerations applied to a true hormone such as testosterone would suggest that many of its biologic effects are achieved by the structurally unaltered molecule. The term "structurally unaltered" as applied here would include the reversible reduction-oxidation of steroids serving a coenzyme function (22). Chemically transformed metabolites might then have their own intrinsic action, if any, with the limitation of these activities imposed by the processes of conjugation and excretion.

The administration of 3β-etiocholanolone was followed by a pronounced increase in the excretion of α-ketosteroids. In Subject W this fraction rose from approximately 17 mg. to 48 mg. following the injection of 100 mg. of 3β-etiocholanolone. Isolation of steroids revealed this increase to consist principally of 3α-etiocholanolone. By means of the isotopic dilution study of this fraction in Subject P (Table 3), a significant conversion of 3β-etiocholanolone to 3α-etiocholanolone was confirmed. Only small amounts of unchanged 3β-etiocholanolone were identified in Subject W (Fig. 4). The small urinary excretion of unchanged 3β-etiocholanolone and the considerable transformation to the 3α-epimer are the major contrasts in the metabolism of these 2 isomeric steroids.

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METABOLISM OF 4-C^{14}-TESTOSTERONE IN HUMAN SUBJECTS
III. FATE OF ANDROSTERONE AND ETIOCHOLANOLONE*

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ABSTRACT

C^{14}-androsterone and C^{14}-etiocholanolone were administered intravenously to 7 human female subjects; 3 received both steroids, and 4 (with bile fistulas) received only one. The radioactivity was excreted in the urine almost quantitatively in a few hours. The unconjugated steroids were cleared from the plasma rapidly, with a half-life of twenty minutes. The level of the glucuronidate fraction of the plasma was approximately ten times higher than that of the unconjugated fraction. The glucuronidates after administration of etiocholanolone were cleared faster than after administration of androsterone. Androsterone and etiocholanolone are not excreted in the bile in appreciable quantities; in 3 of the 4 bile-fistula subjects negligible amounts of radioactivity were recovered. Moreover, after administration of 4-C^{14}-testosterone, no androsterone and only a small amount of etiocholanolone was recovered from the bile. This last observation is tempered by the fact that most of the radioactivity in bile is not extractable after hydrolysis. The results are discussed in relation to the metabolism of C^{14}-testosterone.

INTRODUCTION

WE HAVE previously reported studies on the distribution of radioactivity in bile, blood, feces and urine following the intravenous injection of 4-C^{14}-testosterone (1). These studies were conducted without regard to the chemical form of the radioactivity. Subsequently, in addition to unchanged C^{14}-testosterone, 3 of its metabolites—androsterone, isoandrosterone and etiocholanolone—were isolated and identified from the blood of the subjects following injection. Several other metabolites were isolated in quantities too small to be adequately identified (2). Attempts to isolate metabolites from the bile were partially successful and are de-

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scribed later. The question naturally arises as to whether the radioactivity measured, especially that associated with unconjugated and conjugated steroids in the blood (1), represents injected testosterone or metabolites, or both. One approach to this problem is the injection of labeled metabolites of testosterone, with the objective of ascertaining whether a difference in distribution exists between them and the parent compound. A related problem is the isolation and rigid identification of etiocholanolone in the plasma. Even though this steroid is excreted in the urine in approximately the same amounts as androsterone, endogenous etiocholanolone in contrast to androsterone has not been accurately isolated and identified in the blood of human subjects (3). This has led us to postulate that the threshold for excretion of etiocholanolone may be lower than that of androsterone, thus producing a plasma level so low as to escape detection (2).

Experiments designed to answer some of these questions are the subject of this communication. Data are presented on the clearance of radioactivity from the blood, the rate of excretion, and the distribution of radioactivity in the urine and bile of subjects receiving intravenous injections of the 2 major metabolites of C^{14}-labeled testosterone, namely, C^{14}-etiocholanolone and C^{14}-androsterone.

METHODS AND MATERIALS

C^{14}-androsterone and C^{14}-etiocholanolone were isolated from the urine of subjects given injections of 4-C^{14}-testosterone (1). The specific activity of the androsterone was 220,000 counts/min./mg. and of the etiocholanolone, 206,000 counts/min./mg. No impurities were detected in either compound by infrared spectroscopy or by paper chromatography. Each steroid (2.5–3.9 mg.) was dissolved in 1–2 ml. of absolute ethanol, diluted with 30 ml. of physiologic saline and injected intravenously over a period of one to two minutes. The addition of saline caused the steroid to appear as a fine suspension which did not interfere with its injection.

Seven female subjects were studied. Three, who had undergone mastectomy for carcinoma of the breast four to six weeks prior to the study, received both steroids (C^{14}-androsterone and C^{14}-etiocholanolone), injected at intervals of no less than five days. Four patients, who had T-tube drainage of bile subsequent to cholecystectomy for chronic cholecystitis, received a single steroid. All of the subjects were in good clinical condition without evident renal or hepatic dysfunction. In the cases of biliary T-tube drainage, results of liver function tests were normal preoperatively, and steroid was not injected until three or more days postoperatively when the patient's condition had become stabilized, *i.e.*, there was no fever or other complications, and oral intake of fluids and food had become established. The methods for collection and processing of urine, bile and blood, the determination of radioactivity, and the hydrolytic and extraction procedures have been described in previous publications (1, 4). Throughout this paper the word "unconjugated" refers to steroid metabolites which are extractable with ether or chloroform before performance of any hydrolytic procedures; "glucuronidates" to steroid metabolites which become extractable with ether or chloroform following β-glucuronidase hydrolysis; and "sulfates" to steroid metabolites extracted con-

tinuously with ether for forty-eight hours at pH 1. The methods used for identification of metabolites have been previously described (2, 5).

RESULTS

The data on the clearance of the radioactivity from the plasma following intravenous injection of C^{14}-androsterone and C^{14}-etiocholanolone are presented in Table 1 and summarized in Figures 1 and 2. Blood samples were collected at 15, 30, 60, 120 and 240 minutes after injection. Unconjugated radioactive steroids disappeared from the plasma at a very rapid rate. In fact, at 120 and 240 minutes after injection, the amount of radioactivity was so low as to be difficult to distinguish from that of the background

TABLE 1. RADIOACTIVITY CONCENTRATIONS AS % OF INJECTED DOSE IN TOTAL PLASMA VOLUME IN UNCONJUGATED (U), GLUCURONIDATE (G) AND SULPHATE (S) FRACTIONS OF PLASMA AT VARIOUS TIMES FOLLOWING INJECTION OF C^{14}-ETIOCHOLANOLONE AND C^{14}-ANDROSTERONE

Subject & age (yrs.)	15 min.			30 min.			60 min.			120 min.			240 min.		
	U	G	S	U	G	S	U	G	S	U	G	S	U	G	S
C^{14}-etiocholanolone															
E.F., 57	4.0†	29.3*	2.5*	2.1	24.9	1.9	0.5	12.2	0.8	0.3	9.5	1.4	0.1	4.4	0.2
A.R., 48	3.5†	31.4†	0.5†	1.7	26.8	0.8	0.5	11.7	0.5	0.1	4.8	0.4	0	2.4	0.1
E.E., 59	1.3	18.8	0.2	0.5	13.1	0.2	0.1	7.2	0.1	0	3.3	0	0	1.5	0
E.B., 68	1.6	29.5	0.9	0.9	22.5	0.9	0.6	15.1	0.9	0.3	7.2	0.7	0.2	6.1	0.5
I.N., 24	1.5	28.1	—	1.3	13.6	1.2	0.5	4.3	0	0.1	3.7	0	0.1	1.3	0.2
C^{14}-androsterone															
E.F.	—	24.4	1.5	—	28.9	2.0	—	22.4	0.6	—	16.1	1.6	—	12.3	1.1
A.R.	1.0	29.0	2.1	0.5	26.0	0.7	0.4	24.6	0.4	0.1	17.1	0.4	0	13.2	0.4
E.E.	1.6	23.3	0.4	0.5	16.3	0.6	0.3	10.3	0.5	0	8.3	0.3	0	5.1	0
L.P., 32	5.4	19.5	5.7	2.2	17.6	7.7	1.2	10.1	19.7	0.4	7.3	6.0	0.3	2.6	8.2
D.H., 52	4.2	29.5	1.6	2.1	23.1	0.9	0.9	21.9	1.1	0.7	17.5	2.1	0.3	11.8	2.8

* Sample taken at 8 minutes.
† Sample taken at 10 minutes.

count. Using, then, only the values derived from the first 3 withdrawals of blood after injection, the half-lives of etiocholanolone and androsterone were found to be 19 and 20 minutes, respectively (Fig. 1). For comparison, the disappearance rates of the unconjugated steroids following injection of 4-C^{14}-testosterone (1) and of tetrahydrocortisone (6) were included. The disappearance rate of the latter, with a half-life of 22 minutes, closely matched those of the 2 experimental steroids, whereas the rate for testosterone differed markedly, being slightly faster initially and slower subsequently.

The data on the clearance of the glucuronidates from plasma (Table 1) are summarized and compared with similar data on 4-C^{14}-testosterone (1) in Figure 2. The levels were roughly ten times higher than those for

androsterone and etiocholanolone. Although the concentrations of androsterone and etiocholanolone glucuronidate were nearly identical at fifteen and thirty minutes after injection, the rate of disappearance of the latter was greater than that of the former. This resulted in a significant difference in the levels at 120 and 240 minutes ($.02 < P < .05$), when the level of etiocholanolone glucuronidate was half to one-third that of androsterone glucuronidate.

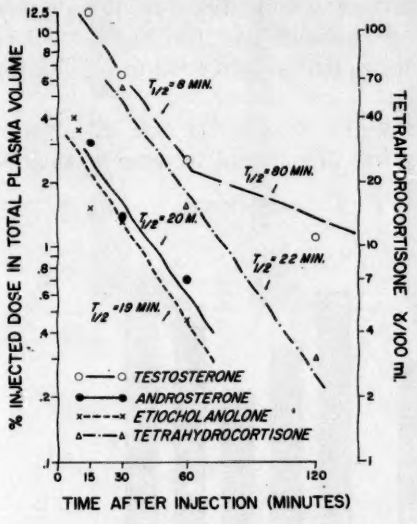

FIG. 1

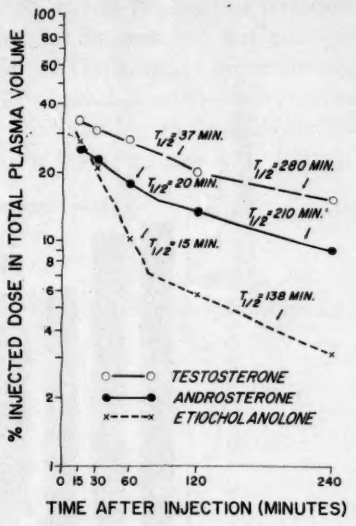

FIG. 2

FIG. 1. The clearance of unconjugated radioactive steroids from the plasma following the intravenous injection of C^{14}-labeled testosterone (1), androsterone and etiocholanolone. The slope with a $T_{1/2}$ of 80 minutes for C^{14}-testosterone is the initial segment of a longer curve published previously (1). The clearance of tetrahydrocortisone is based on previously published data (5). The plasma volume was calculated as 5 per cent of the body weight.

FIG. 2. The clearance of radioactive steroid glucuronides from the plasma of subjects receiving intravenous injections of C^{14}-labeled testosterone (1), androsterone and etiocholanolone.

The plasma concentrations of the sulfate fraction were of the same order of magnitude as those of the unconjugated fraction, with the exception of an unusually large amount in Subject L. P. These levels declined slowly, but somewhat erratically, with time.

Practically all of the extractable radioactivity in the plasma was released by the 3 procedures discussed. By refluxing with 1.5 *N* hydrochloric acid or digestion of the protein residues with hot alkali, as much as an additional 0.8 per cent of injected radioactivity was rendered extractable.

This represented the total radioactivity extractable from all 5 blood samples from a given subject, by both procedures. Again, Subject L.P. had an unusually high value of 6.7 per cent.

The amount of the *total* radioactivity associated with the total red blood cell mass (calculated as 4 per cent of body weight) was very small when compared to that present in the plasma, and constituted on the average less than 0.5 per cent of the injected radioactivity. The highest values were obtained in the blood samples drawn within thirty minutes following the injection of the radioactive steroids.

The recovery of radioactivity in the urine and in the bile is shown in Figure 3. The average total recovery was 97 per cent for etiocholanolone

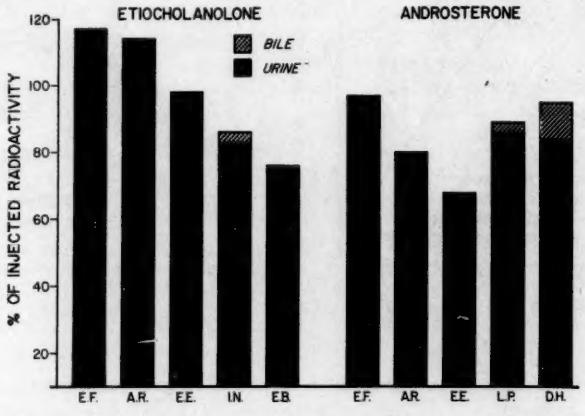

FIG. 3. Excretion of total radioactivity in the urine and bile following intravenous injection of C^{14}-labeled etiocholanolone or androsterone.

and 83 per cent for androsterone. Only small amounts were recovered in the bile—2.7 and 0.7 per cent in the case of etiocholanolone and 2.9 and 11.1 per cent in the case of androsterone. The radioactivity was excreted rapidly in the urine as can be seen from Figures 4 and 5. Usually 50–75 per cent of the radioactivity was excreted in the first four hours and the rest followed at a low, steadily declining rate during the next two days.

Hydrolysis was carried out by boiling the urines with acid as previously described (1), except that the reflux time was reduced to fifteen minutes. Only a portion of the radioactive compounds in the urine were hydrolyzable and extractable by this procedure—on the average, 65 per cent in the case of etiocholanolone and 55 per cent in the case of androsterone. Hydrolysis with β-glucuronidase (1) prior to acid hydrolysis did not yield higher amounts of extractable radioactivity. Gradient elution chromatography (2, 7) revealed that nearly all of the hydrolyzed and extracted radio-

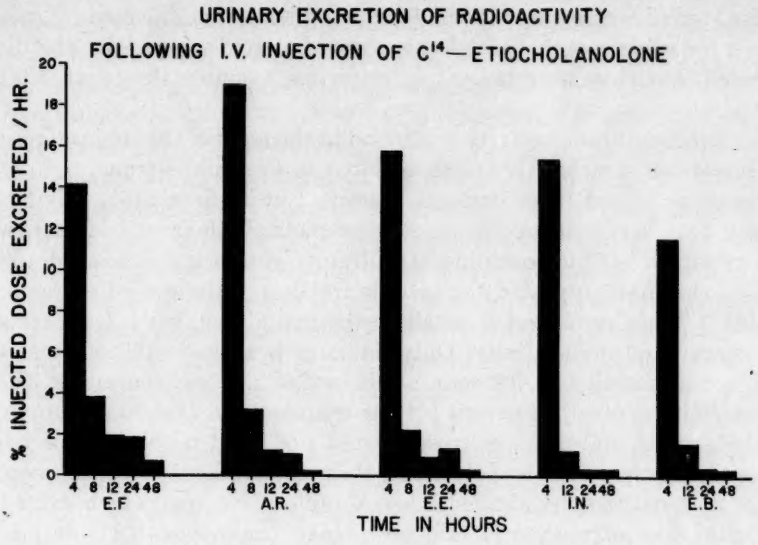

FIG. 4

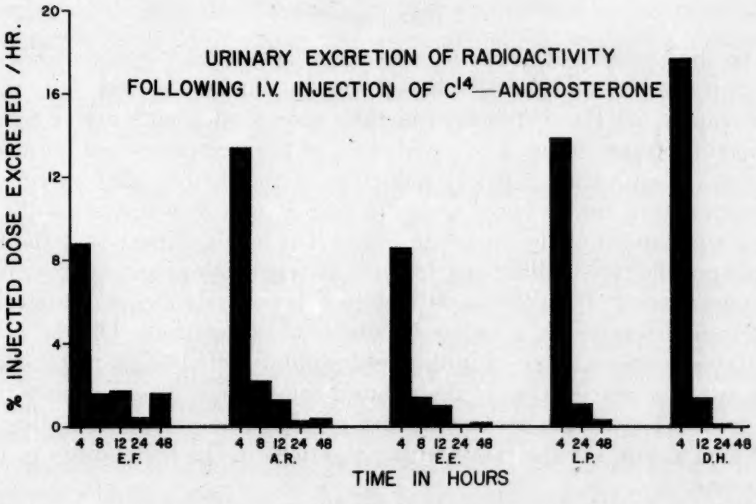

FIG. 5

FIG. 4. The excretion is expressed as the percentage of the dose excreted per hour during successive periods following injection of the steroid.

FIG. 5. As shown also in Figure 4, these steroids are excreted at a very rapid rate during the initial four hours following injection.

active steroid was present in the form in which it was injected. A special search for other metabolites of the injected steroids, especially the diols, revealed that they were present in negligible amounts (less than 1.0 per cent).

No significant radioactivity was found in the feces of the subjects receiving injections of either C^{14}-etiocholanolone or C^{14}-androsterone.

Since the bile of these patients contained insufficient radioactivity for isolation studies, recourse was made to extracts of bile from 4 subjects who had received 4-C^{14}-testosterone (1). Results obtained following gradient elution chromatography of the ketonic fraction of the pooled extracts revealed 1 large peak and 2 small peaks containing both Zimmermann chromogen and radioactivity. Only the large peak had sufficient material (0.27 mg. containing 12,400 counts/min. out of 2.19 mg. containing 23,300 counts/min. applied) to permit further examination. Due to the impurity of the sample, infrared spectroscopy did not yield a reliable spectrum. After a preliminary run and elution of the area of radioactivity, subsequent paper chromatography showed a single radioactive spot which agreed in Rf with that of authentic etiocholanolone (unknown, 0.31; standard, 0.32). The acetylated material also moved at the same rate as authentic etiocholanolone acetate.

DISCUSSION

The data presented not only shed some light upon the metabolism of C^{14}-androsterone and C^{14}-etiocholanolone in human subjects but, more importantly, on the metabolism of their more biologically active parent compound, testosterone. The metabolism of the neutral steroid hormones involves 3 sequential steps: 1) reduction of the double bond in ring A, 2) reduction of the carbonyl group in ring A, and 3) conjugation (8, 9). Upon comparison of the curves in Figure 1 it would appear that the last step is not the rate-limiting one, for both etiocholanolone and androsterone disappear rapidly from the blood with half-lives nearly identical with that of tetrahydrocortisone, a reduced metabolite of cortisone. On the other hand, testosterone, after an initial very rapid distribution, is metabolized at a rate one-quarter that of the reduced compounds. This indicates that the reducing reactions in ring A, and possibly the oxidation of the 17-hydroxyl group, are the rate-limiting reactions in the metabolism of testosterone.

Although androsterone and etiocholanolone appear to be conjugated at nearly identical rates, the glucuronidates are excreted at significantly different rates. In spite of the fact that the levels of the 2 glucuronidates are nearly identical at 15 and 30 minutes after injection (although the data form lines of differing slopes), at 120 and 240 minutes there is a significant

difference, the level for etiocholanolone being the lower. This fact probably explains the failure of others, as well as ourselves, to isolate and identify etiocholanolone in normal human plasma.

Large volumes (5–10 liters) of blood-bank plasma were subjected to hydrolytic and extraction procedures in our laboratory following the addition to the plasma of a small amount of C^{14}-etiocholanolone (10). Gradient elution (7) resulted in the quantitative recovery of the C^{14}-etiocholanolone and identification of endogenous dehydroisoandrosterone and androsterone by infrared spectroscopy, but not in definite identification of endogenous etiocholanolone. The presence of androsterone and dehydroisoandrosterone in human plasma has been previously reported (3, 11). Even though technical factors may account for our failure to isolate etiocholanolone from human plasma, we believe that the explanation presented here is more likely.

Conjugates of androsterone and etiocholanolone do not appear to be the predominant metabolites in bile after the administration of testosterone. This deduction is based mainly on a comparison of the present data with those of Figure 6 in reference No. 1. That figure represents a summary of the biliary excretion of radioactivity in 5 patients who received 4-C^{14}-testosterone. Four of them excreted 11–14 per cent of the injected radioactivity in the bile and the fifth excreted 56 per cent. On the other hand, after administration of androsterone and etiocholanolone, only 0.7–2.9 per cent of the radioactivity was excreted in the bile in 3 of the 4 subjects. If the major metabolites of testosterone in the bile were androsterone and etiocholanolone, one would have expected a substantial fraction (11–14 per cent) of the administered metabolites in these experiments to appear in the bile. Actually, less than one-fifth as much was excreted in the bile, leading to the conclusion that androsterone and etiocholanolone probably are not the major biliary metabolites of testosterone. This thesis is supported by the isolation of only trace amounts (0.2 per cent) of etiocholanolone from the bile of patients receiving injections of C^{14}-testosterone. Androsterone was not identified, although it may have been the smaller peak of radioactivity immediately preceding etiocholanolone on the gradient elution column. These statements, however, can at best be only tentative as long as the majority of the biliary metabolites of testosterone defy analysis. In this connection, it is interesting that Gallagher *et al.* (12) failed to demonstrate any known ketosteroid in the neutral ketonic fraction from the stools of subjects receiving injections of deuterium-labeled etiocholanolone and androsterone. A negligible amount of radioactivity was found in the androsterone and etiocholanolone fractions of the stools after the injection of labeled testosterone (13).

The extremely rapid urinary excretion of radioactivity following intra-

venous injection of C^{14}-androsterone and C^{14}-etiocholanolone is consonant with a negligible entero-hepatic circulation. A similar situation is seen in in the case of Δ^4-androstene-11β-ol-3,17-dione (14) and of cortisol (15).

Some further information regarding the metabolic transformation of C^{14}-testosterone may be gained from the data of the present studies and those of other investigators. It is known that the 2 major identifiable metabolites of testosterone are etiocholanolone and androsterone. Even though the ratio of the latter 2 compounds varies from subject to subject and at different times in the same subject, only about 50 per cent of injected labeled testosterone can be accounted for primarily by these 2 substances. An additional 5–10 per cent can be identified as other metabolites for a total of approximately 60 per cent, despite almost quantitative recovery of the radioactivity in the urine. The question naturally arises whether the missing 40–50 per cent of metabolites of testosterone can be accounted for by further metabolism or unusual conjugation of etiocholanolone or androsterone or by the direct formation of hitherto unknown metabolic products of testosterone. In our series, despite some variation in the amounts of unchanged etiocholanolone or androsterone recovered in the urine, only 55–65 per cent of urinary radioactivity could be attributed to unchanged injected steroid; the remaining 35–45 per cent appeared either as conjugates not hydrolyzable by present procedures, or as extremely hydrophilic metabolites. Even lesser amounts of deuterium-labeled androsterone and etiocholanolone (25 and 50 per cent, respectively) were recovered from urine following injection of these steroids intramuscularly by Gallagher *et al.* (12). Our data and those of the latter authors lend support to the concept that testosterone is primarily metabolized to etiocholanolone and to androsterone, with approximately 50 per cent of the latter 2 steroids undergoing further metabolism and/or conjugation to form water-soluble metabolites.

Our failure to isolate and identify such metabolites of etiocholanolone and androsterone as their corresponding diols, is of interest. Dobriner and Lieberman (16) and West *et al.* (17) showed that the diols accounted for about 5 per cent of the metabolites of large doses of testosterone. Fukushima *et al.* (13) believed that even smaller amounts of the diols were present in the urine of subjects receiving injections of labeled testosterone. In addition, Gallagher *et al.* (12) found that, following the intramuscular injection of 100 mg. of deuterium-labeled etiocholanolone, only 0.4 per cent appeared in the urine as etiocholanodiol. Since they presented no data on the excretion of androstanediol following the injection of 100 mg. of deuterium-labeled androsterone, it is assumed that none was found. Our failure to find significant amounts of the diols of C^{14}-labeled etiocholanolone and androsterone following injection points to the conclusion that

reduction at the 17-keto position *per se* is not an important pathway for the metabolism of these steroids. The substantial conversion of androstane-3α,17α-diol to the 17-keto analog, reported by Dorfman and associates (18), indicates that oxidation at the 17-keto position occurs more readily than reduction. Thus, it would appear that identification of the remaining unknown metabolites of etiocholanolone and androsterone is a problem demanding further investigation.

Acknowledgments

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STUDIES ON THE BIOLOGIC CHARACTERIZATION OF HUMAN GONADOTROPINS

II. COMPARISON OF HUMAN PITUITARY GONADOTROPIN (URINE) AND HUMAN CHORIONIC GONADOTROPIN (URINE)*

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ABSTRACT

Six assays were carried out for human chorionic gonadotropin (HCG) by the method of simultaneous response of the ovary and uterus of the intact immature rat. The constants of these assays were then compared with similar constants derived from assays of human pituitary gonadotropin (HPG) reported earlier. The slope of the ovarian response to HPG was five times that of HCG. The uterine response to HPG was a linear function of log dose; that to HCG was autocatalytic and could be separated into 2 components, each of which was a linear function of log dose. The slope of the uterine response to HPG was not the same as that of either of the 2 limbs of the response to HCG. The dose ratios that integrate gonadotropic action on the ovary and uterus were widely different for HPG and HCG. It was evident that HCG was six times more effective than HPG in inducing the secretion of estrogen by the ovary. It was concluded that HCG and HPG are vastly different in their gonadotropic action, and that the constants of the assay by this biologic system can be used for the characterization and identification of these 2 human gonadotropins.

IT WAS shown previously that the quantitative relationships in the simultaneous response of the ovary and uterus of the intact rat to the administration of human pituitary gonadotropin (HPG) were the same regardless of the source (urine of men, and postmenopausal and castrated women) (1) or the degree of purity (from one- to sixty-fold purification) (2, 3). This suggested a method for characterizing gonadotropins; more specifically, it suggested a technic for distinguishing human pituitary gonadotropin from other human and animal gonadotropins.

The present report is a comparison of HPG and human chorionic gonadotropin (HCG) using the same method of assay. This study was carried

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out because, somewhat to our surprise, pertinent information on the behavior of HCG for comparative purposes could not be found in the literature. Although HCG has been subjected to more biologic testing than any other gonadotropin, we could not find data obtained under the same conditions as those described for HPG, namely the same strain of animal, same dosage schedule, same volume of injection, a comparable range of doses, and the use of the simultaneous response of ovary and uterus as end points.

MATERIALS AND METHODS

Female weanling rats of the Sprague-Dawley strain, ranging in age from 21 to 23 days and in weight from 35 to 50 Gm., were used. HCG was generously supplied by Ayerst Laboratories, New York, N. Y., through the courtesy of Dr. John B. Jewell. This commercial preparation of HCG was checked occasionally against the international standard preparation by the hyperemia method (4). Since general agreement was obtained, the manufacturer's stated potency was accepted. A range of doses from 0.1 to 10 international units per animal was prepared.[1] Each dose was administered to a group of 5 to 10 rats. The volume injected was always 2 ml.; one injection per day was given for four days, with necropsy on the fifth day. This schedule is exactly the same as that used for studies of HPG reported previously.

RESULTS

Figures 1 through 6 show 6 dose-response curves. For the curve in Figure 1, a five-point assay was carried out to insure that the doses employed were in the appropriate range to induce responses in both the uterus and the ovary. For the curves in Figures 2 and 3, an eight-point assay and an 11-point assay, respectively, confirmed the fact that the range of doses from 0.1 to 10 international units was sufficient for the present purpose. These assays also confirmed an initial observation that the uterine response when plotted on semilog paper was biphasic—a point that will be discussed in detail subsequently. For the curve in Figure 4, a 12-point assay with 10 animals per dose was performed with a range of doses from 0.1 to 1.2 international units per animal, to determine more definitely whether the uterine response was a nonlinear function of the log of the dose. On the basis of the data obtained, 2 more assays of 15 and 14 points, respectively, were made (Figs. 5 and 6), the doses being distributed to obtain definitive information on the nature of the ovarian and uterine response.

COMMENT

Although the action of various gonadotropins on the ovary of the rat has been studied in great detail, only meager information exists on the

[1] It is to be emphasized that results of this study relate only to a range of doses from 0.1 to 10 international units of HCG. Parameters of the full dose-response relationship to HCG will be reported elsewhere.

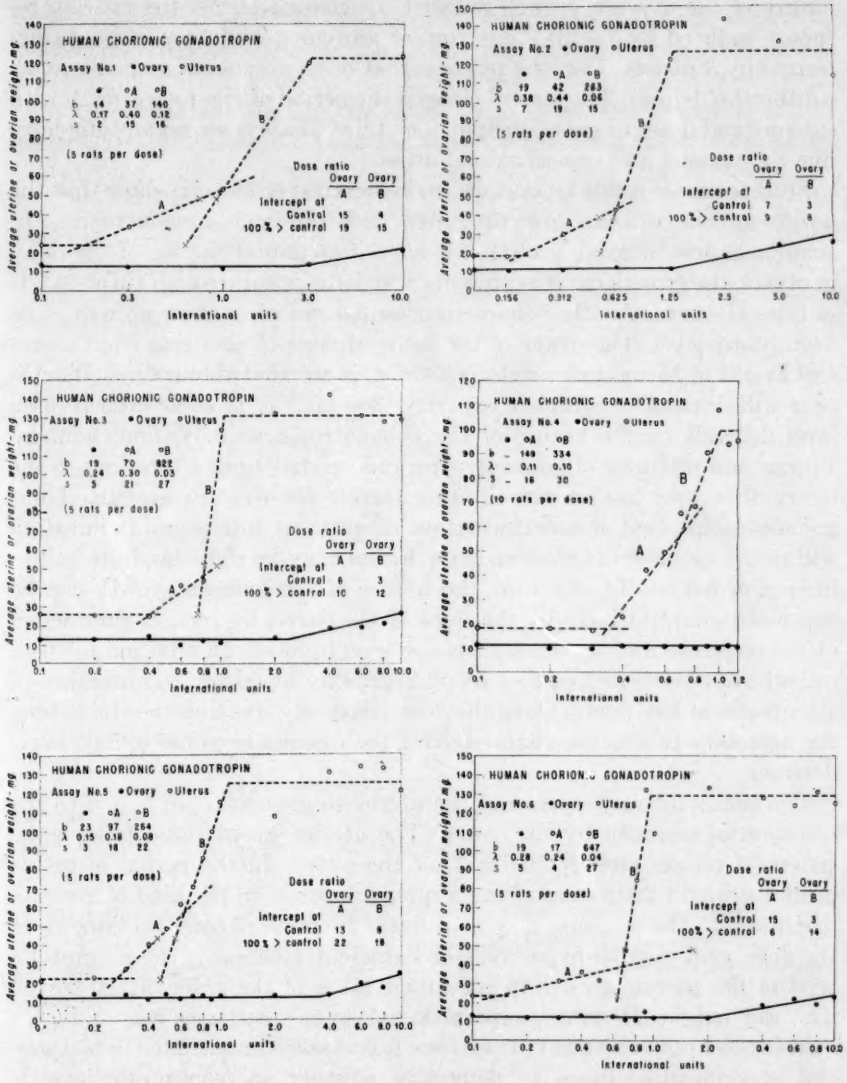

FIG. 1. Five-point assay of human chorionic gonadotropin (HCG). Symbols and values are explained in text; b = slope; λ = index of precision; s = variance.

FIG. 2. Eight-point assay of HCG.

FIG. 3. Eleven-point assay of HCG.

FIG. 4. Twelve-point assay of HCG.

FIG. 5. Fifteen-point assay of HCG.

FIG. 6. Fourteen-point assay of HCG.

nature of the ovarian growth induced. In general terms, the ovarian response induced by "acute" injection of various gonadotropins is characterized by 3 phases. The first phase is that of an absence of response at all subthreshold doses. The second phase is the period of vigorous growth with submaximal doses of gonadotropin. The third phase is an asymptotic level due to maximal and supermaximal doses.

Studies made in this laboratory, to be detailed elsewhere, show that the nature of the ovarian growth varies with different gonadotropins. In some instances, ovarian growth is a linear function of the log of the dose; in others, the growth curve represents a hyperbolic curve or an autocatalytic type of curve. Another characterizing feature of ovarian growth is its asymptotic level. The ovary of the rat is capable of changing from a control weight of 15 mg. to a weight of 300 mg. in a period of four days. Whether it will respond to complete capacity (300 mg.) or to some intermediate level depends on the nature of the gonadotropin used. Various gonadotropins and mixtures of gonadotropins induce structural alterations in the ovary that may act as rate-limiting factors for ovarian growth. Thus, gonadotropins that induce formation of cysts or intensive luteinization will result in curves of ovarian growth that may be different from others in rate or extent. In any case, the nature of the ovarian growth can be expressed quantitatively by the slope of the curve, by certain parameters of the response, and by the asymptotic level induced. In addition to these quantitative measurements of ovarian growth, histologic examination of the ovaries at key points along the dose-response curve aids in establishing the necessary criteria for characterizing the ovarian response to any gonadotropin.

The simultaneous response of the uterus to gonadotropin is due to the secretion of estrogen by the ovary. The uterine growth also follows the general 3 phases already outlined for the ovary. In the period of active uterine growth, the nature of the response depends on the kind of gonadotropin used. The response may be a linear function of some metameter of the dose, or it may be hyperbolic, or sigmoidal. Generally, the asymptotic level of the uterine growth in the intact rat is of the order of 100 to 150 mg., wet weight. However, supermaximal doses sometimes may induce a regression of uterine weight. Therefore, it is of some importance to test several supermaximal doses to determine whether an asymptotic level is reached. For the purpose of these studies, an asymptotic level is assumed to be present when the uterine responses at maximal and supermaximal doses fluctuate randomly about their mean. If the uterine response declines with increasing supermaximal doses, a regression is assumed when the regression constant differs significantly from zero. Thus, it is possible to obtain some information that various gonadotropins may produce secre-

tion of estrogen from different sites in the ovary and that various gonadotropins may induce the secretion of different types of steroids from the ovary.

In these studies, the simultaneous response of the uterus has been quantitatively related to that of the ovary. When the 2 responses are inte-

TABLE 1. COMPARISON OF PARAMETERS OF ASSAY OF HPG (URINE) AND HCG (URINE): MEAN VALUES ± STANDARD ERROR

Parameter*		HPG	HCG	"t"†
Ovarian response	$\bar{b}$	108 ± 5.9	18.8 ± 1.4	12.1
	$\bar{\lambda}$	0.11 ± 0.01	0.24 ± 0.04	
Uterine response				
Single component	$\bar{b}$	247 ± 18.6		
	$\bar{\lambda}$	0.11 ± 0.02		
Double component				
A	$\bar{b}$		68.6 ± 19.7	6.6
	$\bar{\lambda}$		0.28 ± 0.06	
B	$\bar{b}$		412 ± 108	1.5
	$\bar{\lambda}$		0.07 ± 0.02	
Dose ratios				
Single component				
O/U Control		1.7 ± 0.09		
100% > Control		1.8 ± 0.09		
Double component				
O/A Control			11.2 ± 1.9	5.0
100% > Control			13.2 ± 3.1	3.7
O/B Control			4.2 ± 0.9	2.8
100% > Control			13.4 ± 1.7	6.8
Type of curve				
Ovarian		Linear, log dose	Linear, log dose	
Uterine		Linear, log dose	Autocatalytic	
Asymptotic response, mg.				
Ovary		300	‡	
Uterus		112	122	

* $\bar{b}$ = Mean slope; $\bar{\lambda}$ = mean index of precision; A and B = limb A and B of uterine curve of HCG; O = ovary; U = uterus.

† Student's "t" test.

‡ The full dose-response curve for HCG will be reported elsewhere.

grated, a method for characterizing various gonadotropins evolves. In each of 13 previously reported (1) assays of HPG from various sources, ovarian growth and uterine growth were linear functions of the log of the dose. Because of our special interest in the nature of HPG, dose-response curves of all gonadotropins were plotted on semilog paper despite the fact that some gonadotropins induce responses which are not linear functions of the log dose.

As seen in Figures 1 through 6, a semilog plot of the uterine response to HCG shows 2 linear limbs. In reality, the uterine curve is an autocatalytic curve showing no discontinuity, as can be determined by plotting the results on arithmetic co-ordinates. Since each limb is now a linear function of the log of the dose, calculation of the parameters of the assay for HCG is rendered easy. This was carried out according to methods previously described (5). These parameters are shown in Table 1 and are compared with similar ones obtained from the curves for HPG (1). It is evident that the slope of the ovarian response to HPG is five or more times greater than that to HCG. The slope of the uterine curve for HPG does not approximate that of either of the 2 limbs of the HCG curve. The dose ratios,[2] calculated as described previously (1), are widely different. For HPG, the dose ratio of 1.7 (ovary/uterus at control intercept) means that the threshold dose for the uterus is 0.6 ($1 \div 1.7$) that of the ovarian threshold dose. The comparable dose ratio (11.2) for limb A of the HCG curve means that the uterine threshold dose is about 0.1 ($1 \div 11.2$) that of the ovarian threshold dose. On this basis, HCG is, therefore, six times ($0.6 \div 0.1$) more effective than is HPG in inducing secretion of estrogen by the ovary.

For purposes of visual comparison, it is necessary to derive a composite curve for HCG and HPG. In Table 2 are listed the actual values in international units taken from each of the curves (Figs. 1–6). For the ovary, 2 entries are given, namely the dose in international units at which the ovarian response intercepts the control ovarian weight, usually 15 mg., and the dose in international units at which a doubling of the control weight of the ovary occurs, usually 30 mg. Similar values were obtained for the intercept of limb A of the uterine curve at the control weight (usually 25 mg.), for the dose at which a doubling of uterine weight occurred, for the intercept of the B limb at the control weight of 25 mg., for the dose at which a doubling in uterine weight occurred on the B limb, for the dose at which the A and B limbs intersected, and finally for the dose at which the asymptotic level of the uterine response was reached. Also

[2] The values for the mean dose ratios given in Table 1 are mean values from the intercepts read from each of the 6 curves. The values for the mean dose ratios shown in Figure 7 are slightly different, because of exclusion of 2 entries, as stated in Table 2. The values given in Figure 7 are, therefore, more accurate.

TABLE 2. VALUES OF HCG IN INTERNATIONAL UNITS FOR INTERCEPTS OF OVARIAN AND UTERINE DOSE-RESPONSE LINES*

Exp. No.	Ovarian		Uterine						Uterine asymptote (mg.)
			A		B		A/B	B, max.	
	At 0	At 100%	At 0	At 100%	At 0	At 100%			
1	2.6	15	.17	.75	.62	.93	1.00	3.10†	122
2	1.3	4.5	.18	.49	.51	.60	0.63	1.30	126
3	2.4	10.0	.40	.95	.75	.80	0.80	1.05	131
4	—	—	.35	.47	.50	.59	0.70	1.00	100
5	3.5	10.0	.26	.45	.46	.56	0.64	1.10	125
6	2.0	10.0	.14	1.60‡	.63	.70	0.68	0.95	129
Mean	2.4	10.0	.25	.65	.58	.69	0.73	1.1	122
÷2.4	x	4.2x	.1x	.27x	.24x	.29x	.3x	.46x	

* At 0 = intercept at control level; at 100% = intercept at double control weight; A and B = limbs A and B of uterine curve; A/B = intersection of limbs A and B; B max. = dose for maximal, or asymptotic, response of uterus.

† This value was not included in the mean because of its obvious inaccuracy due to the large dose interval used in the first (range-finding) assay.

‡ This value was not included in the mean because the low slope of the A limb in this assay introduces a large error in extrapolating this intercept.

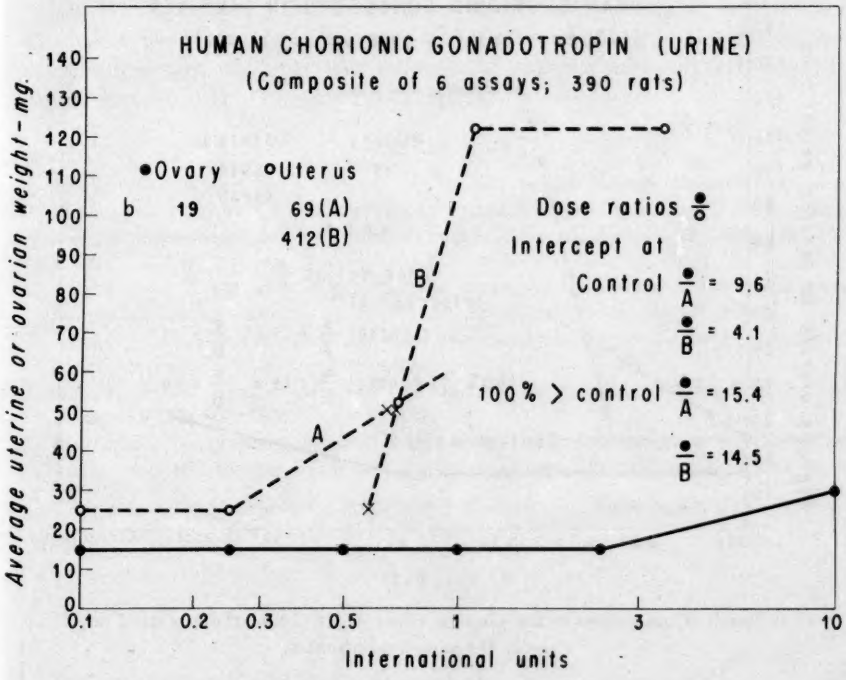

FIG. 7. Summary curve for the assay of HCG. The average values in international units are from Table 2.

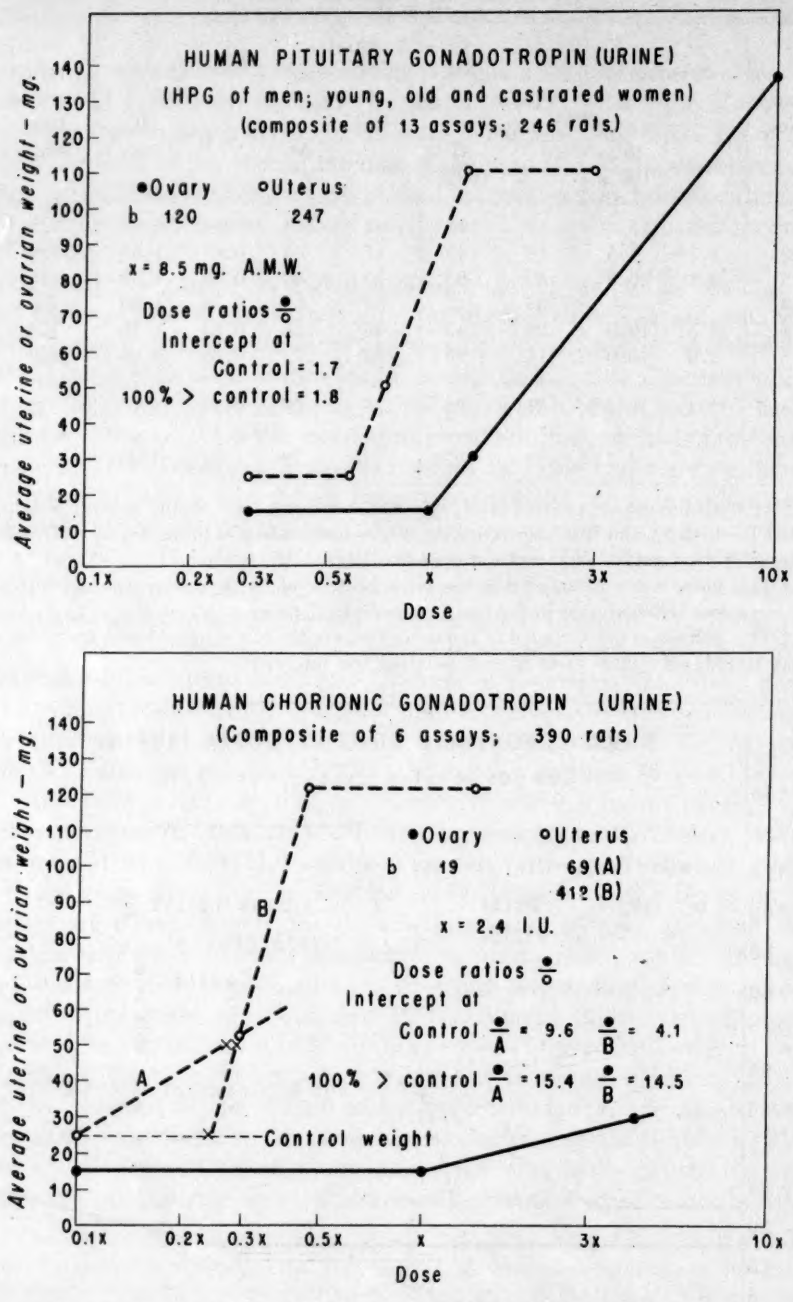
HUMAN PITUITARY GONADOTROPIN (URINE)
(HPG of men; young, old and castrated women)
(composite of 13 assays; 246 rats.)
•Ovary ○Uterus
b 120 247
x = 8.5 mg. A.M.W.
Dose ratios •/○
Intercept at
Control = 1.7
100% > control = 1.8
Average uterine or ovarian weight – mg.
Dose
0.1x 0.2x 0.3x 0.5x x 3x 10x
HUMAN CHORIONIC GONADOTROPIN (URINE)
(Composite of 6 assays; 390 rats)
•Ovary ○Uterus
b 19 69(A)
41?(B)
x = 2.4 I.U.
Dose ratios •/○
Intercept at
Control •/A = 9.6 •/B = 4.1
100% > control •/A = 15.4 •/B = 14.5
A
B
Control weight
Average uterine or ovarian weight – mg.
Dose
0.1x 0.2x 0.3x 0.5x x 3x 10x

FIG. 8. Comparison of the biologic effect of HCG and HPG plotted on exactly the same co-ordinates.

shown in Table 2 and plotted in Figure 7 are the asymptotic levels of the uterine weight (milligrams) read from each curve. Each of these latter entries is a mean of all the responses induced by supermaximal doses in any one assay.

The pivotal point for comparison of all types of gonadotropin is the intercept of the ovarian response at its control level. The mean value for the ovarian intercept was 2.4 international units and was, therefore, designated as x. This value was divided into the remaining mean values. This yields fractions or multiples of x for all other intercepts. These fractions or multiples can then be plotted to obtain a composite curve. This curve is automatically the composite curve for the action of HCG, the respective slopes of which are equal to or extremely close to the mean of slope values derived from the individual curves. Figure 8 is a comparison of the composite curve for HCG when transformed in terms of x with a composite curve similarly obtained for the aforementioned 13 assays of HPG.[3] The 2 gonadotropins are now compared in exactly the same coordinates. It is obvious that these 2 gonadotropins differ greatly in their effect on the simultaneous response of the ovary and uterus. The difference is so large that the shape of these curves can be used for purposes of identification. Were an investigator given 2 unlabeled samples, disguised with inert material so as to be of equal weight, one of which was HCG and the other HPG, correct identification could be made easily by injecting a graded series of appropriate doses. Calculation of a few of the parameters or a simple plot of the results on semilog paper would correctly identify which sample is HCG and which is HPG.

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[3] The ovarian slope is given as 120, which is the mean of a much larger series (97 slope determinations) reported elsewhere (3).

THE SECRETION AND DISPOSITION OF CORTISOL DURING PREGNANCY*

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ABSTRACT

The following findings were obtained which bear on the mechanisms concerned in the rise of plasma free 17-hydroxycorticoid (17-OHCS) levels during pregnancy: 1) Cortisol accounted for approximately 90 per cent of the plasma free 17-OHCS in pregnant women during the third trimester. 2) No cortisol (F), cortisone (E), tetrahydrocortisol (THF) or tetrahydrocortisone (THE) were detected in the urine of 2 adrenalectomized pregnant patients maintained on 1 mg. of 9-alpha-fluorohydrocortisone daily during the third trimester. 3) When ACTH was administered intravenously to a woman with intact adrenals during pregnancy and the postpartum period, there was a greater response during pregnancy as determined by the excretion of F+E and the rise in the level of plasma free 17-OHCS, but no greater response as determined by the excretion of F+E+THF+THE. 4) In a normal woman followed throughout pregnancy there was a marked rise in plasma free 17-OHCS levels but little change in urinary total 17-OHCS excretion. 5) When cortisol was administered intravenously to an adrenalectomized woman, there was a diminished rate of removal of free 17-OHCS from the plasma and a smaller apparent distribution volume of the injected F during the third trimester as compared to the postpartum period. The first of these findings was reproduced by one week's administration of estrogen to a woman who was receiving prednisone therapy.

It is concluded that the rise in the level of plasma cortisol during pregnancy is due mainly to a diminished rate of transformation of F to certain of its metabolites and to a greater retention of the hormone within the intravascular compartment.

Two factors are described which may prevent the development of more marked signs of Cushing's syndrome during pregnancy: first, a diurnal variation in plasma free 17-OHCS levels such that for part of the day the levels are within the normal range; and second, a greater retention of cortisol within the intravascular compartment.

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THE plasma levels of free 17-hydroxycorticoids have been found to rise progressively during pregnancy (1–4). The present report describes attempts to elucidate the mechanisms involved in this rise and gives consideration to the possible biologic consequences of this phenomenon.

METHODS

Plasma free 17-hydroxycorticoids (17-OHCS) were measured by the method of Peterson, Karrer and Guerra (5). The normal range for this method is 6–25 μg. per 100 ml.

Urinary total 17-hydroxycorticoids were determined by the modified Reddy, Jenkins and Thorn procedure (6). The normal range for this procedure is 1–10 mg. per twenty-four hours.

Estimation of cortisol (F), cortisone (E), tetrahydrocortisol (THF) and tetrahydrocortisone (THE) in urine. Duplicate urine samples were adjusted with 50 per cent sulfuric acid to a pH of approximately 5.0. Then 12.5 ml. per 100 ml. of urine of 4 *M* acetate buffer (pH 4.9) and 50,000 units per 100 ml. of urine of β-glucuronidase ("Ketodase," Warner-Chilcott Laboratories) were added and incubation was carried out at 47° C. for twenty-four hours. The product was adjusted to pH 1 with 50 per cent sulfuric acid and extracted twice with one volume of ethylene dichloride. The extracted urine was incubated a second time with β-glucuronidase and again extracted twice with one volume of ethylene dichloride. The ethylene-dichloride extracts were combined and first washed with one-tenth volume of 5 per cent sodium bicarbonate, then with one-tenth volume of distilled water. The extract was dried over anhydrous sodium sulfate, filtered, and taken to dryness in vacuo at 45° C. in a flash evaporator. The dried extract was dissolved in 8 ml. of benzene and extracted four times with 20 ml. of distilled water. The aqueous extract was taken to dryness in the flash evaporator as described, and the residue was dissolved in 20 ml. of 30 per cent ethanol. The ethanolic extract was then washed three times with 5 ml. of the petroleum ether (boiling range 38–48° C.). The ethanolic extract was taken to dryness in the flash evaporator and the residue dissolved in 10 ml. of methanol and transferred with washing (2×5 cc. methanol) to a conical centrifuge tube. The methanolic extract was dried with suction in a 50° C. water-bath. Prior to chromatography it was redissolved in 1.0 ml. of methanol.

The sheets of Whatman No. 1 filter paper (6×24 inches) which were used for chromatography had previously been washed by descending flow in a chromatography cabinet, with distilled water for three days and with methanol for four days. The sheets of filter paper were divided into 3 rows, 1 row for application of 10 μg. each of crystalline F, E, THF and THE dissolved in methanol, and 2 rows for application of duplicate samples of the methanol extract of urine. The standards and urine extracts were applied with a capillary pipette and dried with a stream of air. The chromatograms were run using the Bush (7) system, benzene:methanol:water (2:1:1), and the Zaffaroni (8) system, toluene:propylene-glycol. The former system was run for four hours for all 4 compounds; the latter system was run for six to seven days for THF and THE and for sixty hours for F and E. On completion of the chromatography run, the papers were air-dried for twenty-four hours and ultraviolet photographs were taken on Kodagraph contact paper using a General Electric 15-watt germicidal lamp with over 95 per cent of its output at 256 mμ. The row on the chromatogram containing the standards and 1 of the 2 rows containing the urine extract were then sprayed with a solution containing 2 parts of 2 per cent blue tetrazolium in water and 1 part of 10 per cent sodium hydroxide. The

areas of paper on the remaining urine-extract row which corresponded to the spots of the F, E, THF and THE standards, were then cut out. Two sections of paper of a size identical to those containing the steroids were obtained from areas of the chromatogram which did not react with blue tetrazolium; these were used as blanks. The sections of filter paper removed from the chromatogram were cut into squares measuring approximately 0.5 cm. in diameter and allowed to stand overnight in 20 ml. of methanol. The eluates were then filtered under suction through fritted discs and the squares of filter paper were washed three times with 20 ml. of methanol. The methanol extract was dried in the flash evaporator and the residue dissolved in 95 per cent ethanol. The steroid content of the ethanolic solutions was then determined by the method of Izzo, Keutmann and Burton (9). Extensive experiments had indicated that the average loss of steroid during elution from the paper was 20 per cent; correction was therefore made for this loss. It should be noted that the F, E, THF and THE separated from the urine extracts were partly characterized on the basis of their having running rates on the chromatograms similar to those of the authentic standards, in both the benzene:methanol:water and the toluene:propylene-glycol systems. The compounds were further characterized according to their ability to produce both the Porter-Silber and blue tetrazolium reactions. F and E were also detected by their ability to absorb ultraviolet light under the photographic conditions described—a quality not possessed by THF and THE.

PROCEDURE AND RESULTS

I. *The nature of the plasma free 17-hydroxycorticoids (17-OHCS) during pregnancy*

The method (5) which was used in the present study for the estimation of plasma free 17-OHCS utilized the Porter-Silber colorimetric reaction. This reaction is relatively specific for 17,21-dihydroxy-20-ketosteroids, a group which includes cortisol, cortisone, 11-desoxy-17-hydroxycorticosterone (Reichstein's compound S) and their dihydro- and tetrahydroderivatives. It became important for the present study to determine the nature of the compound or compounds measured by the free 17-hydroxycorticoid method in the plasma of pregnant women. For this purpose a plasma pool of 300 ml. was obtained from 31 normal women who were in the third trimester of pregnancy. The plasma was processed according to the free 17-hydroxycorticoid procedure (5). When the methylene-chloride extract had been purified, 10-ml. aliquots were taken for estimation of 17-OHCS, using cortisol as the standard. The remainder of the extract was evaporated to dryness, and the residue dissolved in methanol and chromatographed on paper, as described for urine extracts under Methods. The blue tetrazolium reaction was seen in only 2 spots. One spot was characterized (in the manner described under Methods) as cortisol, the other as cortisone. The 2 substances were eluted from the chromatogram and estimated by the Porter-Silber reaction (6), using cortisol as the standard. The results, expressed in micrograms per total volume of methylene-chloride extract, were as follows: (a) free 17-OHCS as determined on the methylene-chloride extract prior to chromatography, 63.3 μg.; and (b)

17-OHCS eluted from the chromatogram—cortisol 56.9, cortisone 8.6, total 65.5 μg. From these results it may be concluded that cortisol accounts for approximately 90 per cent of the free 17-OHCS measured by the method of Peterson *et al.* (5) in the plasma of pregnant women during the third trimester. Similar findings have recently been reported by Migeon and his co-workers (10), who used the Nelson and Samuels (11) method for measurement of plasma free 17-OHCS.

II. *Do the placenta and the fetal adrenal cortex secrete cortisol into the maternal circulation?*

An opportunity to answer this question arose with the occurrence of pregnancy in 2 women who had previously undergone complete adrenalectomy for Cushing's syndrome. The case reports of these patients, Mrs. E. K. and Mrs. N. B., have already been published (12).

Mrs. E. K. was studied during the thirty-second week, and Mrs. N. B. during the thirty-eighth week of pregnancy. For the period of the study, cortisol or cortisone therapy was discontinued and the patients were maintained on 2 mg. of 9-alpha-FF for the first day and on 1 mg. of 9-alpha-FF daily thereafter. On the third day following cessation of cortisol or cortisone therapy a 24-hour urine was collected on ice for determination of F, E, THF and THE. None of these compounds could be detected in the urine of either of the 2 adrenalectomized pregnant women. With the experimental procedure used, the minimum amount of each compound which could have been detected was 30 micrograms per day. In normal pregnant women the average total amount of these 4 components of the urine during the third trimester is of the order of 10 mg. per day (13)—a level some one hundred times the total amount which could have been detected in the present experiments. These findings suggest that little or no cortisol was released into the maternal circulation from the placenta or the fetal adrenal in these patients and that one must seek elsewhere for the major source of the increase in plasma cortisol levels during pregnancy. While this work was in progress, similar findings were reported by Baulieu, Bricaire and Jayle (14). They detected only 50 μg. of THF and 50 μg. of THE per day and no F or E in the urine of a pregnant Addisonian patient whose cortisone therapy was withdrawn during the third trimester.

III. *Does the maternal adrenal cortex secrete increased amounts of cortisol during pregnancy?*

An indirect approach to the solution of this problem was made by studying the response to intravenous administration of adrenocorticotropin (ACTH) in a 23-year-old woman during the third trimester of pregnancy and again six months post partum.

Mrs. S. L. This patient was first admitted to the Toronto General Hospital in January 1956, with a history of recurrent renal calculi during the preceding four years. A diagnosis of renal hyperchloremic acidosis was made, on the basis of an elevated concentration of serum chloride (112 mEq/L.), lowered serum carbon-dioxide combining power (20 mEq/L.), and an inability to acidify the urine below pH 5.8 following administration of ammonium chloride. Renal function studies revealed the following: normal level of blood nonprotein nitrogen (30 mg. per 100 ml.), normal urinary concentrating power (specific gravity of urine reached 1024 after twenty-four hours of fluid restriction), normal inulin clearance (112 ml. per minute), slightly diminished p-aminohippuric acid (PAH) clearance (407 ml. per min.), considerably diminished Tm PAH (26 mg. per min.), and a normal phenolsulfonphthalein excretion (48 per cent in fifteen minutes and 82 per cent in two hours). The patient was given alkali therapy in the form of a solution of sodium citrate, potassium citrate and citric acid (containing 1 milliequivalent of sodium, 1 milliequivalent of potassium and 1 millimole of citrate per ml.) in a dosage of 25 ml. four times daily by mouth. With this therapy the hyperchloremic acidosis was corrected and the tendency to produce renal calculi was completely halted.

In July 1956 the patient became pregnant for the first time (last menstrual period June 28; expected date of confinement April 5, 1957). During the pregnancy the patient continued alkali therapy in the same dosage. On several admissions to the hospital, no evidence of a return of her primary disease was detected. She gained 25 pounds during the pregnancy. There was no edema or elevation of blood pressure. On April 10, 1957 a healthy female infant, weighing 7 pounds 3 ounces, was delivered following a six-hour labor.

The patient has been followed at regular intervals during the ten months following delivery. She has continued to take the same amount of alkali therapy and no signs of recurrence of the hyperchloremic acidosis have been noted. The authors are grateful to Dr. E. R. Yendt for permitting them to study this patient.

TABLE 1. URINARY EXCRETION OF F, E, THF AND THE IN MRS. S. L. FOLLOWING INTRAVENOUS ADMINISTRATION OF ACTH (8 A.M.–4 P.M.) DURING THE THIRD TRIMESTER (38TH WEEK OF PREGNANCY) AND AGAIN SIX MONTHS POST PARTUM

Steroid	Urinary steroid excretion (mg./period)							
	8 A.M.–12 noon		12 noon–8 P.M.		8 P.M.–8 A.M.		Total	
	3rd tr.	p.p.	3rd tr.	p.p.	3rd tr.	p.p.	3rd tr.	p.p.
F	0.42	0.27	2.83	1.39	0.45	0.13	3.70	1.79
E	0.07	0.10	0.79	0.39	<0.1	0.16	0.86	0.65
THF+THE	1.33	1.93	8.23	13.10	6.50	6.60	16.06	21.63
F+E+THF+THE	1.82	2.30	11.85	14.88	6.95	6.89	20.62	24.07
$\frac{F+E}{THF+THE}$	0.39	0.19	0.44	0.14	0.07	0.04	0.28	0.11

The ACTH was administered to Mrs. S. L. during the thirty-eighth week of pregnancy and again six months post partum in the following way: a dose of 25 U.S.P. units of Armour corticotropin in 500 ml. of 5 per cent dextrose in water was given intravenously from 8 A.M. to 4 P.M. Blood was drawn for determination of plasma free 17-OHCS at appropriate times before, during, and following the infusion of ACTH. Urines were collected on ice for the periods 8 A.M. to 12 noon, 12 noon to 8 P.M., and 8 P.M., to 8. A.M. They were analyzed for their content of F, E, THF and THE. The results of the plasma and urinary analyses are shown in Table 1 and Figures 1 and 2. It can be seen that the capacity to re-

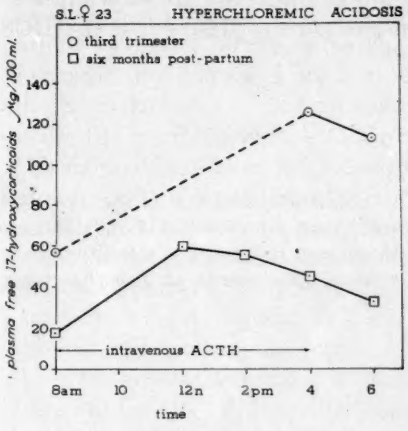

FIG. 1. Effect of intravenous ACTH (25 U.S.P. units) on the level of plasma free 17-OHCS in Mrs. S. L. during the 38th week of pregnancy and again six months post partum.

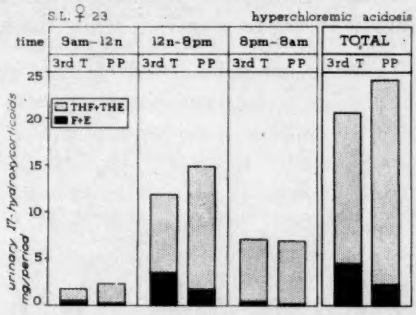

FIG. 2. Effect of intravenous ACTH (25 U.S.P. units, 8 A.M.–4 P.M.) on urinary excretion of F+E and THF+THE in Mrs. S. L. during the third trimester (38th week of pregnancy) and again six months post partum.

spond to the 25-unit dose of ACTH as measured by urinary excretion of F+E+THF+THE was, if anything, somewhat greater in the postpartum period than in the third trimester. However, the response to the ACTH as determined by a rise in the level of plasma free 17-OHCS and by urinary excretion of F and E was considerably greater in the third trimester than in the postpartum period. A similar hyperactive response to ACTH in pregnant women during the third trimester (as measured by a rise in the level of plasma free 17-OHCS) has already been reported by Christy, Wallace and Jailer (15).

A possible interpretation of these findings is that in pregnancy there is a *diminished* degree of transformation of F and E to THF and THE but a *normal* or even *decreased* secretion of F in response to ACTH. Another

interpretation is that there is an *increased* secretion of F in response to ACTH and that the diminished urinary excretion of THF and THE is the result of *increased* transformation of F to other metabolites such as the 20-hydroxy derivatives of F, E, THF and THE—substances which were not measured in these experiments.

Whatever may be the correct interpretation, the foregoing findings are not necessarily pertinent to the question of whether there is increased secretion of cortisol *under resting conditions* during pregnancy. Evidence which bears on this question was obtained from a study of the levels of plasma free 17-OHCS and urinary total 17-OHCS in a normal 26-year-old woman throughout pregnancy. The results of this study are shown in Figure 3. It can be seen that while the concentration of plasma free 17-OHCS

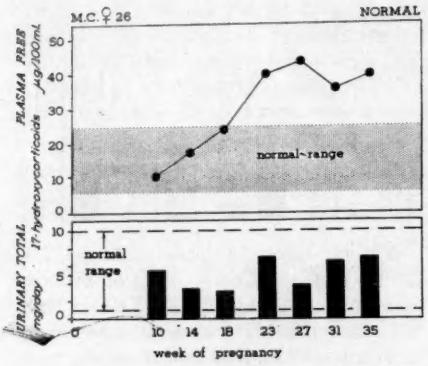

FIG. 3. Plasma levels of free 17-OHCS and urinary excretion total 17-OHCS levels throughout pregnancy in a healthy young woman. Blood was drawn on each occasion at 9 A.M.

gradually increased to levels which were well above the normal range, urinary total 17-OHCS excretion showed little change. Similar findings with regard to urinary total 17-OHCS in pregnancy have been reported by Devis (16). Martin and Mills (17) likewise obtained similar results using the urinary total 17-ketogenic steroid method. The principal steroids measured by this procedure are F, E, THF and THE and their 20-hydroxy derivatives. Martin and Mills observed a very slight increase in urinary 17-ketogenic steroid levels, which did not, however, rise above the normal range. These findings, taken in conjunction with those concerning cortisol secretion in response to ACTH, suggest: (a) that there is little, if any, increase in cortisol secretion from the maternal adrenal cortex, and (b) that the rise in the level of plasma free 17-OHCS is due, in part at least, to a diminished ability of the pregnant woman to transform cortisol to certain of its metabolites. Further evidence favoring this thesis is presented in the following section.

IV. *The disposition of cortisol during pregnancy*

A study of the disposition of cortisol was carried out in 1 of the adrenalectomized patients, *Mrs. N. B.* During the thirty-ninth week of pregnancy and again two weeks and three months post partum this patient was given an intravenous injection of 100 mg. of cortisol (as its hemisuccinate derivative[1]) dissolved in 2 ml. of normal saline. On each occasion the cortisol was administered over a period of one minute, at 8 A.M. Plasma free 17-OHCS levels were determined at 30, 90, 180, and 360 minutes following injection. Prior to each period of study the patient had been maintained on 20 mg. of cortisol and 0.2 mg. of 9-alpha-FF by mouth daily. On each of two days preceding the day of intravenous administration of cortisol, the patient was given 1.0 mg. of 9-alpha-FF only. The results of this study are illustrated in Figure 4. It can be seen that the levels of plasma free 17-OHCS reached at a given time following injection of cortisol were highest during the third trimester and lowest three months post partum.

Using the zero intercept values (Fig. 4), the "apparent" distribution volume (ADV) of the injected cortisol was calculated as described by Samuels and his co-workers (18) and found to be 52 per cent of body weight in the third trimester (body weight, 60.5 Kg.), 71 per cent two weeks post partum (body weight, 54.2 Kg.) and 97 per cent three months post partum (body weight, 54.2 Kg.). It is of interest that the ADV of the administered cortisol was lowest in the third trimester, a period during which the plasma volume is known to be increased to some 30 per cent above normal (19). This finding suggests that there may be a greater retention of cortisol within the intravascular compartment during pregnancy, a phenomenon which might occur if cortisol were bound to a greater extent to the plasma proteins.

A similar study was performed during the thirty-second week of pregnancy and seven months post partum in *Mrs. S. L.*, the patient with intact adrenals, who was the subject of the ACTH-study described in Section III. It can be seen from Figure 5 that the results were similar to those obtained in the adrenalectomized patient (Mrs. N. B.).

Similar findings regarding the rate of removal of injected cortisol from plasma were reported recently by Migeon, Bertrand and Wall (20), who administered *tracer amounts* of C^{14}-cortisol to normal women in late pregnancy. In addition, these workers observed that following the injection of cortisol there was a larger amount of radioactivity in the chloroform ex-

[1] It had been planned to use the free form of cortisol for these experiments. However, in Patient N.B., a reaction occurred following the intravenous administration of an ethanolic solution of free cortisol and it was therefore decided to use the water-soluble hemisuccinate derivative.

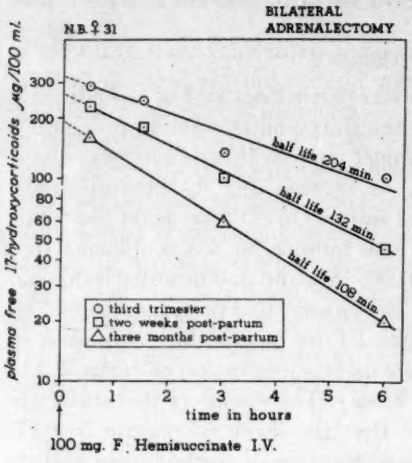

FIG. 4

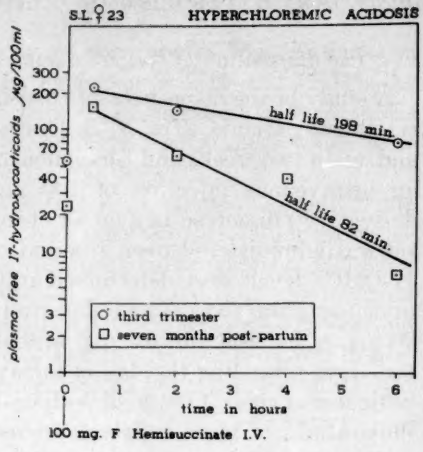

FIG. 5

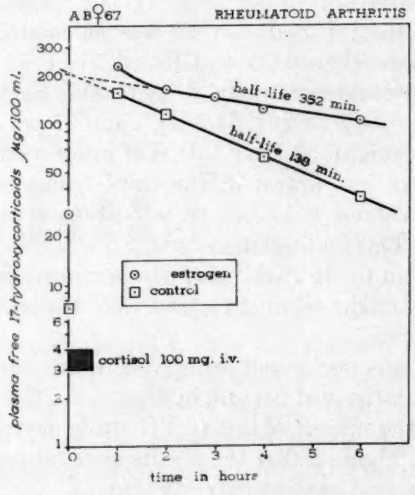

FIG. 6

FIG. 4. Plasma free 17-OHCS levels following intravenous administration of 133.7 mg. of sodium succinate ester of cortisol (equivalent to 100 mg. cortisol) to an adrenalectomized woman during the 39th week of pregnancy and again two weeks and three months post partum.

FIG. 5. Plasma free 17-OHCS levels following intravenous administration of 133.7 mg. of sodium succinate ester of cortisol (equivalent to 100 mg. cortisol) to a woman with intact adrenals during the 32nd week of pregnancy and again seven months post partum.

FIG. 6. Effect of administration of estrogen (0.4 mg. of ethinyl estradiol daily for seven days) on plasma free 17-OHCS levels following intravenous administration of 100 mg. of cortisol (free alcohol) to a patient with rheumatoid arthritis receiving 10 mg. of prednisone daily.

tracts of plasma and urine ("free" fractions) of pregnant women than in those of normal subjects; conversely the radioactivity found in the chloroform extracts of plasma and urine following glucuronidase hydrolysis ("glucuronoside" fractions) was greater in the normal subjects than in pregnant subjects. These findings of Migeon and his co-workers would appear to rule out an altered renal handling of cortisol as an important factor in the elevation of plasma free 17-OHCS levels during pregnancy.

Evidence which indicates that the alteration in disposition of cortisol during pregnancy is related, in part at least, to increased secretion of estrogen, was provided from the following experiment.

Mrs. A. B. was a 67-year-old woman with rheumatoid arthritis and osteoporosis of the spine who had been receiving 10 mg. of prednisone daily for over two years. During the period of study she was given 2.5 mg. of prednisone by mouth every six hours (at 6 A.M., 12 noon, 6 P.M. and 12 midnight). On one day during the control period and on the seventh day following the institution of estrogen therapy (0.1 mg. of ethinyl estradiol by mouth at 6 A.M., 12 noon, 6 P.M. and 12 midnight) the patient was given 100 mg. of cortisol in 250 ml. of 5 per cent dextrose in water containing 10 ml. of ethanol. The solution containing the hormone was administered intravenously from 8 to 8:30 A.M., and plasma free 17-OHCS levels were determined at 8 A.M., 9 A.M., 10 A.M., 12 noon and 2 P.M. The results of this study are illustrated in Figure 6. It can be seen that administration of estrogen produced a decrease in the rate of removal of the injected cortisol from plasma that was similar in degree to that observed during pregnancy in Mrs. N. B. (Fig. 4). Calculation (from the zero intercepts of the 2 curves in Figure 5) of the apparent distribution volume (ADV) of the infused cortisol revealed a volume of 73 per cent of body weight during the control period (body weight, 65.5 Kg.) and 76 per cent following administration of estrogen (body weight, 64.5 Kg.). This finding indicated that administration of estrogen for seven days in Mrs. A. B., unlike pregnancy in Mrs. N. B., produced little or no change in the ADV of the injected cortisol. It may also be noted from Figure 6 that, following administration of estrogen, there may have been a slower achievement of distribution equilibrium of the infused cortisol. In the case of Mrs. A. B. it is assumed that, as a result of long continued prednisone therapy, there was little endogenous production of cortisol, and hence the effect of estrogen was due largely to an alteration in disposition of the injected cortisol and perhaps in small degree to a change in the disposition of the exogenous prednisone. A similar decrease in the rate of removal of injected cortisol from the plasma of patients with *intact adrenals* who were given ethinyl estradiol was described recently by Wallace and her associates (21). It is also of interest that both diethylstilbestrol (22) and ethinyl estradiol (21) were

shown to produce a rise in plasma free 17-OHCS levels in patients with intact adrenals who were not receiving corticosteroids.

V. *Biologic consequences of the increased level of plasma free 17-OHCS in pregnancy*

Bayliss and his co-workers (2) demonstrated that by the fifth month of pregnancy the majority of women have levels of plasma free 17-OHCS which are above the normal range (see also Fig. 3). Thereafter the levels remain high, or rise still further to levels which during the third trimester may exceed those of many patients with Cushing's syndrome. In view of these findings it might be asked why pregnant women do not show more of the signs and symptoms associated with this disease. In an attempt to provide an answer to this question it was decided to determine whether pregnant women, unlike patients with Cushing's syndrome ((23) and Figure 9), show a diurnal variation in plasma free 17-OHCS levels. For this purpose determinations were made at 8–9 A.M. and 4–5 P.M. on 6

TABLE 2. PLASMA FREE 17-OHCS LEVELS AT 8–9 A.M. AND 4–5 P.M. IN 6 HEALTHY PREGNANT WOMEN

Patient and age (yrs.)	No. of previous pregnancies	Length of gestation (wks.)	Plasma free 17-OHCS (μg./100 ml.)	
			8–9 A.M.	4–5 P.M.
M.D., 20	0	17	16.0	12.6
P.F., 32	2	26	35.0	18.5
J.H., 26	0	27	38.5	21.8
M.Cr., 37	0	28	38.2	19.6
R.V., 31	3	32	42.4	26.3
E.B., 24	0	34	28.2	14.2

healthy women in various stages of pregnancy (Table 2), and at appropriate times throughout the day and night on 4 other healthy women (H. K. aged 28, M. C. aged 26, L. L. aged 34, and E. R. aged 31) who were respectively, 21, 31, 37 and 38 weeks pregnant (Figs. 7 and 8). Patient E. R. was studied in a similar manner four days after delivery. It can be seen from Table 2 that in all 6 women of the first group the values at 4–5 P.M. were not only lower than those at 8–9 A.M. but were either at the upper limit of normal or well within the normal range. The results depicted in Figures 7 and 8 indicate that the pregnant woman has a diurnal pattern of plasma free 17-OHCS levels similar to that described for normal subjects by Samuels and his associates (18), *i.e.*, with the lowest concentration between 12 midnight and 4 A.M., a rapid rise between 4 A.M. and 8 A.M.,

and a tendency towards a gradual decrease in levels throughout the day and early evening. In Figures 7 and 8 it can also be noted that the diurnal variation in plasma free 17-OHCS levels is such that for at least part of the day the levels are well within the normal range.[2] The degree to which 2 women at approximately the same stage of pregnancy may differ in this regard is shown in Figure 7; in Mrs. E. R. (38 weeks) all values but that at 3 A.M. were *above* the normal range, whereas in Mrs. L. L. (37 weeks) all

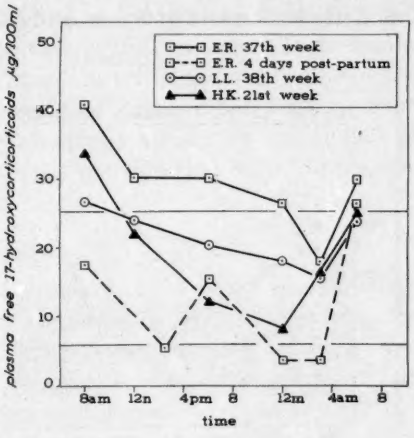

FIG. 7. Diurnal pattern of plasma free 17-OHCS levels in 3 healthy women. The normal range (6–25 µg. per 100 ml.) is represented by the 2 horizontal lines.

values but that at 8 A.M. were *within* the normal range. On the basis of the 9 A.M. values for plasma free 17-OHCS in Mrs. M. C. (Fig. 8), it would be most unlikely that signs of Cushing's syndrome could appear before the eighteenth week of pregnancy. Thereafter she could have been at least partially protected against the development of this disease if she had had a diurnal pattern in plasma free 17-OHCS levels similar to that which she exhibited during the thirty-first week of pregnancy.

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[2] It should be emphasized that this normal range was largely derived from determinations on bloods drawn between 8 and 10 A.M. In view of the diurnal variation in plasma free 17-OHCS levels, it is likely that the normal range of values differs somewhat throughout the day and night. Hence, it would be more correct to state that the diurnal variation in plasma free 17-OHCS levels in pregnancy is such that for at least part of the day the levels are within the *8 to 10 a.m. normal range*. Despite this phenomenon, it is likely that for a given subject the plasma free 17-OHCS levels at all times of the day and night are higher in the third trimester of pregnancy than in the non-pregnant state (see 2 curves on Patient E.R. in Figure 7). Nonetheless, the pregnant woman differs from the patient with Cushing's syndrome, who not only has a high level of plasma free 17-OHCS at 8 A.M. but, because of loss of diurnal variation, maintains this high level throughout the 24-hour period.

Indirect evidence that there may be other, and perhaps more important, factors which protect the pregnant woman against the development of marked signs of Cushing's syndrome was provided from the following study:

Mr. W. C. was a 71-year-old man with carcinoma of the prostate who had undergone bilateral orchiectomy. Diethylstilbestrol in a dosage of 10 mg. daily had been started approximately one year previously. At the time of study the patient was without symptoms and was receiving 5 mg. of diethylstilbestrol at 8 A.M. and at 8 P.M. daily. There was a hard, irreg-

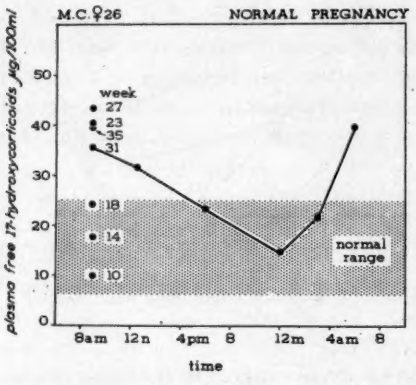

FIG. 8

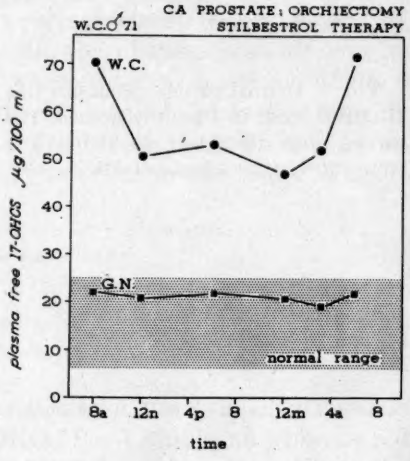

FIG. 9

FIG. 8. Plasma free 17-OCHS levels at 9 A.M. in various stages of pregnancy and throughout the day and night in the 31st week of pregnancy in a healthy young woman.

FIG. 9. Diurnal pattern of plasma free 17-OHCS levels in a man (Mr. W.C.) with carcinoma of the prostate who had been receiving estrogen therapy for over a year. For comparison, a similar study in a woman (Mrs. G.N.) with Cushing's syndrome due to bilateral adrenal hyperplasia is shown.

ular enlargement of the prostate but no clinical or laboratory evidence of metastases; there was no clinical evidence of Cushing's syndrome. Urinalysis findings were normal, blood non-protein nitrogen concentration was normal, and urinary total 17-OHCS excretion averaged 3.3 mg. per day (a *normal* value). The study consisted of the drawing of blood at appropriate intervals during the day and night for measurement of plasma free 17-OHCS levels. The results are illustrated in Figure 9.

A similar study (Fig. 9) was performed preoperatively in *Mrs. G. N.*, a 39-year-old woman with Cushing's syndrome who was found at operation

to have bilateral adrenal hyperplasia. Preoperatively this patient had a slightly elevated urinary excretion of 17-OHCS (average 10.6 mg. per day) and manifested a hyperactive response to intravenous ACTH.

It can be seen in Figure 9 that in the woman with Cushing's syndrome, the plasma free 17-OHCS levels, although only in the upper portion of the normal range, did not show the normal decline throughout the day and early evening (range of values 18.5–22.0 μg. per 100 ml.). On the other hand, in the man receiving estrogen therapy who showed no signs of Cushing's syndrome, there was a diurnal variation in plasma free 17-OHCS levels similar in pattern to that observed in both normal and pregnant subjects. However, the levels were at all times considerably above the normal range. In this patient, it is evident that factors other than a diurnal variation in plasma free 17-OHCS levels largely accounted for the lack of development of Cushing's syndrome. It is possible that such factors also exist in pregnancy.

DISCUSSION

The findings of the present study indicate that the rise in plasma levels of cortisol during pregnancy is *not* due, appreciably, to secretion from the placenta or fetal adrenals or to an increase in secretion of the hormone from the maternal adrenal cortex. Rather this rise is mainly due to 2 other factors: 1) a diminished ability of the pregnant woman to transform cortisol to certain of its metabolites, particularly tetrahydrocortisol and tetrahydrocortisone, and 2) an alteration in the distribution of cortisol in pregnancy, resulting in greater retention of the hormone within the intravascular compartment—as suggested from the study of the disposition of injected cortisol in Mrs. N. B. (Fig. 4).

The first of these 2 factors has been recognized recently by Migeon and his associates (10, 20). That it is related to the increased secretion of estrogen in pregnancy is suggested by the finding (in Mrs. A. B.) that administration of estrogen greatly diminished the rate of removal of injected cortisol from the plasma. This view is further supported by Glenn (24), who observed inhibition of the degradation of cortisol by the rat-liver microsome supernatant fraction in the presence of estradiol.

That the second of the 2 factors—alteration in the distribution of cortisol in pregnancy—may be related to increased estrogen secretion is not ruled out by the results of the present experiments, but it is of interest that such an alteration was not produced in Mrs. A. B. by a seven-day course of estrogen therapy. It is conceivable that this change may be due to a binding of a larger amount of cortisol to plasma proteins, a phenomenon which has been described for thyroxine in pregnancy by Dowling, Freinkel and Ingbar (25).

In the light of the foregoing remarks, one can discuss other evidence which bears on the mechanisms concerned in the rise of plasma cortisol levels during pregnancy. Jailer (26) reported a progressive rise in the concentration of plasma free 17-OHCS during pregnancy in an Addisonian patient maintained with a constant dose of cortisone. Hills and his associates (27), in a study of a pregnant adrenalectomized patient receiving constant cortisone therapy, found an increase in urinary glycogenic corticoids (presumably mainly cortisol and cortisone) similar to that described by Venning (28) for healthy pregnant subjects. These observations are not at variance with the findings of the present report and can be explained as readily by an alteration in the metabolism of administered cortisone as by secretion of corticosteroids from an extra-adrenal source. It is possible that the small quantities of corticosteroids which were isolated by Johnson and Haines (29) and by de Courcy, Gray and Lunnon (30) from extracts of human placenta could have arisen from the blood contained in the placental tissue.

One factor which may prevent the development of marked signs of Cushing's syndrome in pregnant women has been described. This is the presence of a diurnal variation in plasma cortisol levels which is similar in pattern to that observed in non-pregnant subjects. It would seem reasonable that there is an associated diurnal variation in tissue cortisol levels. If this be so, it is possible that in pregnancy the tissue cortisol levels do not remain above normal for a sufficiently long period during the day and night to produce marked clinical changes. One other factor, however, has been described which may protect the pregnant woman against the development of marked signs of Cushing's syndrome. This is a greater retention of cortisol within the intravascular compartment. If this phenomenon were to result in a smaller proportion of the hormone reaching the tissues, then a given plasma cortisol level would be associated with less biologic activity in pregnant women than in normal subjects. In this connection it is of interest that a pregnant Addisonian patient who was maintained with a constant dose of cortisone was not protected against the development of acute adrenocortical insufficiency when a mild dental infection supervened (27). Further, it has been shown by Beaton and Curry (31) that 15 mg. of cortisone per day given to pregnant rats had less effect on blood glucose and amino-nitrogen levels than one-third of this dose administered to nonpregnant female animals.

Englert and associates (32) showed that in patients with cirrhosis of the liver there is defect in the metabolism of cortisol which is similar to that described here for pregnant women. However, in cirrhotic patients the resting levels of plasma free 17-OHCS are normal, whereas in pregnant women they are elevated. It would appear that during pregnancy the

pituitary-adrenal homeostatic mechanism is impaired. The evidence presented in this report suggests the possibility that the increased levels of plasma cortisol in pregnancy are associated with less inhibition of the output of ACTH by the pituitary than are similar levels in normal subjects.

Acknowledgments

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DEPRESSION OF SERUM AND URINARY CITRIC ACID LEVELS BY 17-HYDROXY-CORTICOSTEROIDS*

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ABSTRACT

When the dietary intake is constant, the daily variations in serum and urinary citric acid levels are slight. In this study, during a constant dietary regimen, increased serum concentrations of 17-hydroxycorticosteroids were associated with significantly decreased serum levels and urinary excretion of citric acid. This decrease was observed prior to, or in the absence of any significant changes in calcium, potassium, sodium or acid-base balance which might otherwise explain the direction of the change in citric acid levels. The decrease also occurred despite an increase in urinary calcium excretion or an increase in potassium retention. It is postulated that 17-hydroxycorticosteroids depress serum and urinary levels of citric acid as a consequence of a decrease in the conversion of pyruvic acid to acetyl-CoA (which becomes coupled with oxalo-acetic acid to form citric acid).

THE concentration of citric acid in serum and in urine is known to be altered by changes in acid-base (1, 2) and potassium balance (3) and urinary calcium excretion (4, 5). The relation of these factors to the cellular metabolism of citric acid remains obscure, as does the source and biologic significance of urinary citrate. The data to be reported suggest that 17-hydroxycorticosteroids (hydrocortisone and cortisone) decrease the serum concentration and renal excretion of citric acid. The observed changes in citric acid metabolism during steroid therapy may be more directly related to the effects of these hormones on carbohydrate metabolism (6) than to the effects on acid-base, potassium or calcium metabolism.

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METHODS

Serum and urinary citric acid were measured by the method of Natelson, Pincus and Lugovoy (7) as modified by K. A. C. Elliott (8). Urinary samples were kept refrigerated during and after collection, and determinations were made on aliquots of 24-hour samples or of two- or three-day pools. There was no loss of citric acid in urine stored for three months, if the aliquots were acidified with either an equal volume of 11 per cent trichloroacetic acid or 2 per cent by volume concentrated hydrochloric acid. Venous blood was drawn without stasis after an overnight fast, allowed to clot, and the serum proteins precipitated with cold 11 per cent trichloroacetic acid within thirty minutes of collection. Serum filtrates could be stored at 4°C. for seventy-two hours without loss of citric acid. All patients received constant dietary intakes and were under the conditions of a complete balance study except as noted.

RESULTS

In hyperadrenocorticism

With a constant dietary intake, daily fluctuations in the levels of serum and urinary citric acid were minimal in all subjects. However, under these conditions the observed differences between normal and hyperadrenocortical subjects were statistically highly significant (Table 1).

During administration of ACTH

A 7-month-old infant[1] with hypoglycemia apparently due to hyperinsulinism was studied, under constant dietary conditions, during and after administration of large doses of adrenocorticotropin (ACTH). The 24-hour

TABLE 1. SERUM AND URINARY CITRIC-ACID LEVELS IN NORMAL SUBJECTS AND IN PATIENTS WITH INCREASED BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS, WHILE RECEIVING A CONSTANT DIETARY INTAKE

	Serum citric acid (mg./100 ml.)		Urinary citric acid (mg./24 hrs.)	
	Normal subjects	Cushing's syn. or steroid Rx	Normal	Cushing's syn. or steroid Rx
No. of patients	7	5	7	5
No. of observations	14	15	21	33
Mean	*2.3*	*1.6*	*480*	*254*
Standard Error	*0.21*	*0.23*	*42*	*15*
*P value**		*<.001*		*<.001*

* Significance of observed differences between mean serum and urinary levels in normal and hyperadrenocortical groups.

[1] Studied through the courtesy of Dr. John Crigler, Children's Hospital and Harvard Medical School, Boston, Massachusetts.

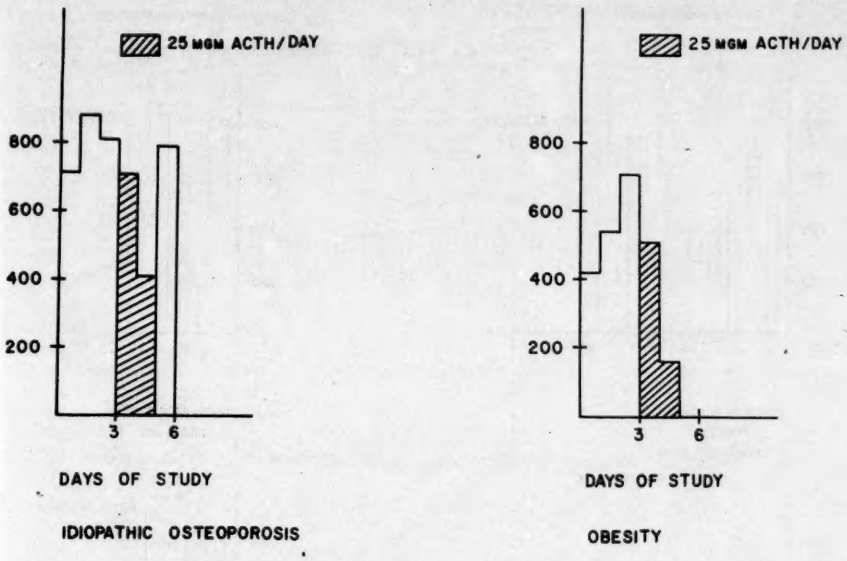

FIG. 1. Urinary citric acid (mg./24 hours) following intravenous infusion of 25 mg. of ACTH over eight hours on two consecutive days, in 2 patients with normal adrenocortical function. (One had idiopathic osteoporosis and the other was obese.)

urinary excretion of citric acid ranged from 0 to 10 mg. during ACTH therapy, and from 95 to 100 mg. three months after withdrawal of ACTH. The level of serum citric acid rose from 1.4 mg. per 100 ml. during ACTH therapy to 3.66 mg. per 100 ml. after withdrawal of ACTH.

The administration of ACTH (25 mg. infused intravenously over eight hours on each of two consecutive days) produced a precipitous fall in the urinary excretion of citric acid to subnormal levels in 1 case of idiopathic osteoporosis and in 1 case of obesity (Fig. 1). In both of these cases, the urinary 17-hydroxycorticosteroid and calcium responses to ACTH were normal. Potassium excretion was not measured in these studies, but one would except it to be increased during the first two days of administration of ACTH (9).

A patient with decreased adrenocortical function secondary to subtotal bilateral adrenalectomy for Cushing's syndrome received an infusion of 25 mg. of ACTH over eight hours on each of two consecutive days. The urinary excretion of 17-hydroxycorticosteroids was in the low-normal range (2.45–2.49 mg. per twenty-four hours) prior to ACTH, and there was only a slight increase (to 4.73–4.88 mg.) during administration of ACTH. With this rise of urinary 17-hydroxycorticosteroids towards the normal range, urinary citric acid decreased from levels above normal (710–830 mg. per twenty-four hours) to normal values (445 mg.).

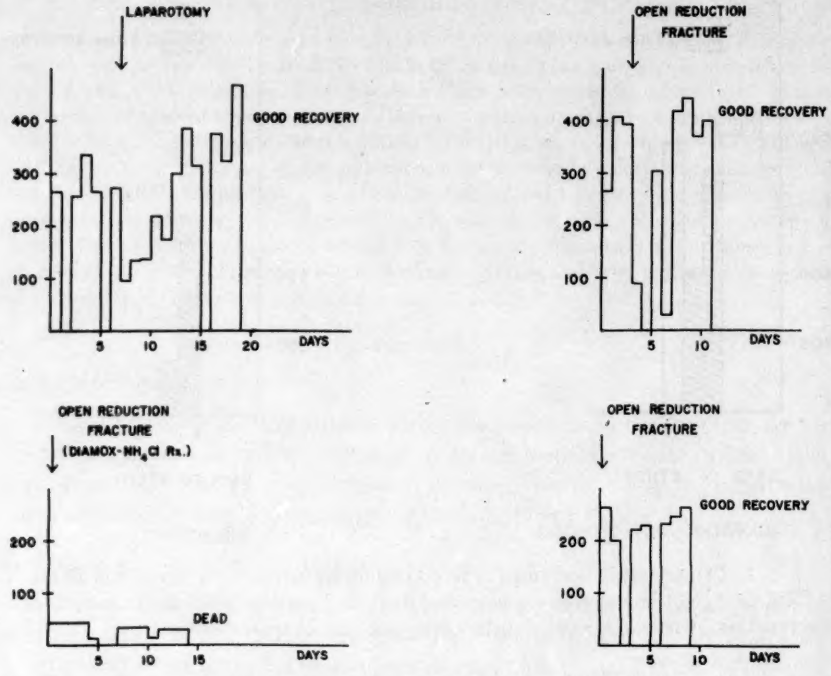

FIG. 2. Urinary citric acid excretion (mg./24 hours) in 4 cases of trauma. One patient had undergone laparotomy and 3 had had fractures treated by open surgical reduction.

Following trauma

As shown in Figure 2, urinary citric acid decreased after laparotomy, and after fractures treated by open surgical reduction. These 4 patients[2] received measured, but not constant dietary intake. Recovery following open reduction of fracture in 2 patients was associated with a gradual return of urinary citric acid to normal levels. The third patient with a fracture failed to recover and urinary citric acid remained extremely low. Moore (10) has shown that the hyperadrenocorticism of initial alarm may persist or recur in patients with extensive tissue damage or complications of the original injury. During the last five days of the study of the third fracture patient, the picture was complicated by congestive heart failure, which necessitated the administration of Diamox and ammonium chloride —agents know to reduce urinary citric acid (1, 2, 11).

[2] Studied through the courtesy of Dr. Francis D. Moore, Department of Surgery, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Massachusetts.

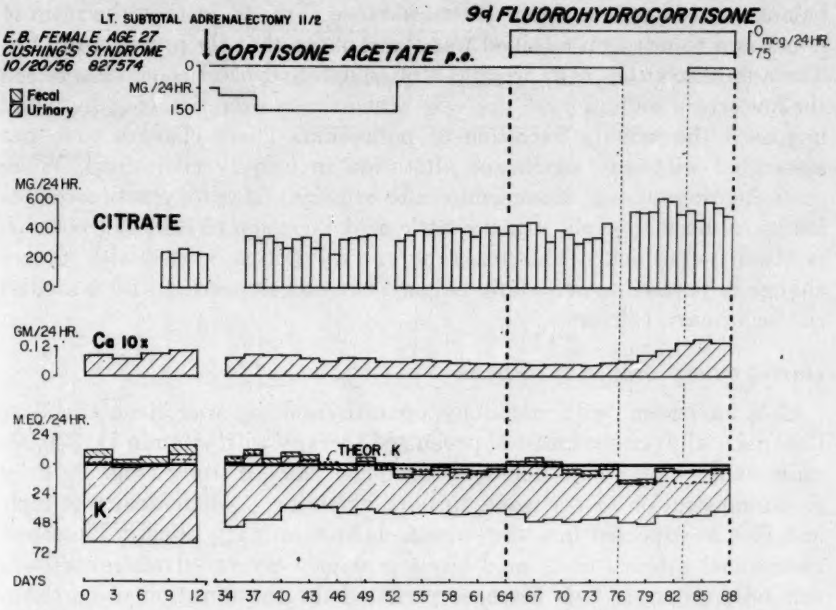

FIG. 3. Urinary excretion of citric acid and calcium, and potassium balance data, in Patient E. B. following curative subtotal adrenalectomy for recurrent Cushing's syndrome.

The potassium intake is charted down from the heavy zero line. Urinary and fecal excretion are charted up from the intake line. A hatched area above the zero line indicates negative balance; a clear area below the zero line indicates positive balance. For explanation of "theoretical K" balance shown by dotted line, see text. The dosage of 75 μg. of 9-alpha-fluorohydrocortisone daily would be expected to effect sodium and potassium balances, but it would have virtually no hydrocortisone-like activity.

Following subtotal adrenalectomy in Cushing's syndrome

The average urinary excretion of citric acid was less than 225 mg. per twenty-four hours prior to surgery in a 27-year-old woman (E.B.) with recurrent Cushing's syndrome (Fig. 3). Following curative subtotal adrenalectomy there was a progressive rise in citric acid excretion. During cortisone therapy (150 mg. per day) urinary excretion of citric acid rose to 350 mg. per twenty-four hours. During this same period there was no significant change in either calcium excretion or potassium balance. Following reduction of the dosage of cortisone to 50 mg. per day, urinary citric acid rose to 380–400 mg. per twenty-four hours. During this phase there was no change in calcium balance, but the balances of potassium and nitrogen became strongly positive. The "theoretical" potassium

balance agreed well with the actual balance (Fig. 3), in that the ratio of potassium to nitrogen retained was the same as that for protoplasm (12). The administration of 75 μg. of 9-alpha-fluorohydrocortisone daily raised the low serum sodium level, decreased the urinary excretion of sodium and increased the urinary excretion of potassium. These changes were not associated with any significant alteration in urinary citric acid. When cortisone therapy was discontinued and urinary 17-hydroxycorticosteroids fell to subnormal levels, urinary citric acid excretion rose to 550–600 mg. per twenty-four hours. This final rapid rise was not associated with further change in potassium or sodium balance but was associated with a modest rise in urinary calcium.

During administration of cortisone

C. V., a patient with pseudohypoparathyroidism, was given cortisone (200 mg. daily) in the midst of prolonged therapy with vitamin D (300,000 units daily) under conditions of a complete balance study (Fig. 4). Prior to administration of cortisone, urinary excretion of citric acid was high, and rose as expected in a step-wise fashion as urinary calcium increased. Serum and urinary citric acid levels promptly decreased when cortisone was administered. This decrease persisted for the duration of cortisone therapy and for two days thereafter. Urinary calcium continued to rise during cortisone therapy. Following withdrawal of cortisone, the values for serum and urinary citric acid rose—the latter to a level higher than the pre-treatment control value.

The changes in citric acid were not associated with marked or persistent changes in sodium or potassium balances during cortisone therapy. Urinary sodium decreased when cortisone was discontinued, and at this time urinary citric acid increased. Potassium excretion increased slightly throughout cortisone therapy, and there was slight retention of potassium for three days following withdrawal of cortisone. However, the theoretical potassium balance agreed well with the actual potassium balance (Fig. 4) except during the three-day period following withdrawal of cortisone. Nevertheless, at this time of potassium retention, urinary citric acid was at its lowest.

DISCUSSION

The present data do not provide direct information concerning the sources of citric acid, nor do they indicate whether the observed changes were due to changes in production or utilization of citric acid. A similar depression of citric acid metabolism by 17-hydroxycorticosteroids has been reported in rabbits (13) and rats (14).

A previous study of intermediary carbohydrate metabolites in the

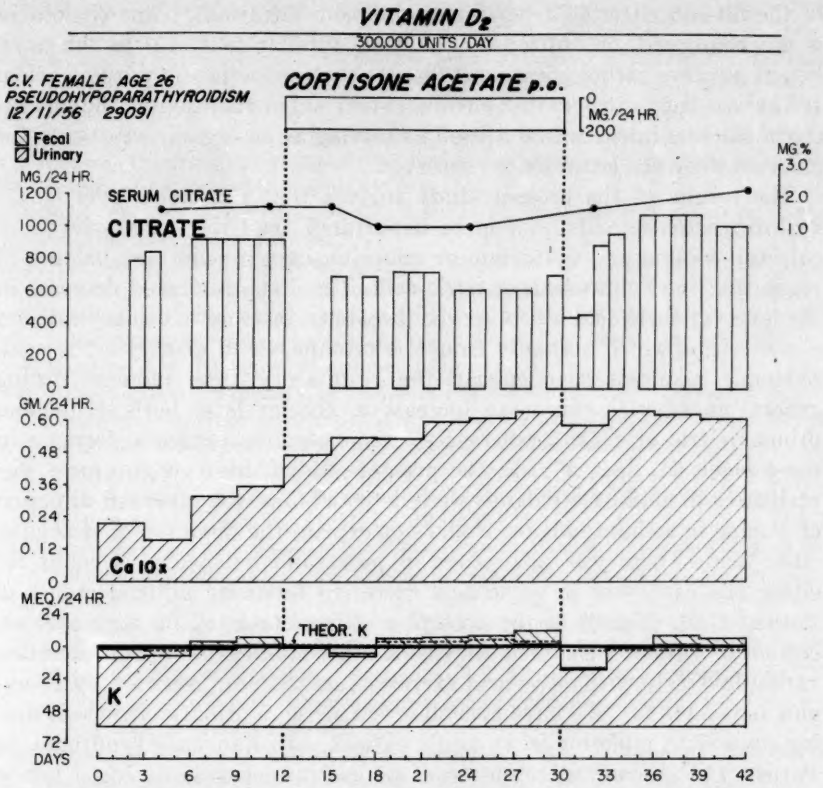

FIG. 4. Urinary and serum citric acid levels during administration of cortisone in a patient (C. V.) with pseudohypoparathyroidism receiving vitamin D therapy. (See Figure 3 for explanation of charting.)

peripheral blood of patients with Cushing's syndrome under free dietary conditions (6) demonstrated a decreased serum concentration of citric acid. This observation lacked rigorous statistical proof, however, due to daily variations in the citric acid content of blood samples from the same patient (fasting). Under the constant metabolic conditions of a complete balance study, as in the present investigation, these day-to-day variations became less marked and serum and urinary levels were statistically significantly below normal in patients with Cushing's syndrome and in patients treated with adrenal steroids ($P < .001$).

The parallelism of serum and urinary citric acid levels suggests that the urinary changes reflect but do not induce the changes in serum. The 24-hour urinary excretion of citric acid represents approximately one-fifth

of the filtered citric acid (assuming complete filtration). Thus citric acid is not conserved, or utilized by the renal tubular cells (15) to the same extent as other carbohydrate metabolites such as lactic and pyruvic acids. It has been suggested (4) that citric acid may aid in maintaining the solubility of calcium in the urine, as well as serving as an organic substitute for chloride when the latter ion is conserved.

The results of the present study suggest that the blood level of 17-hydroxycorticosteroids is of more importance than the urinary levels of calcium, sodium and potassium or minor changes in acid-base balance in regulating the metabolism of citric acid. The steroid-induced decrease in the levels of citric acid was observed despite an increase in urinary calcium —a situation which normally induces a prompt rise in urinary citric acid. Systemic alkalosis (particularly the respiratory type induced during general anesthesia) causes an increase in the levels of both serum and urinary citric acid (16), whereas systemic acidosis causes a decrease in these levels (1, 2, 3, 17). In the present studies, however, no gross disturbances in acid-base balance were detected, and the observed direction of change in sodium balance would not explain the direction of change in citric acid. There was no change in potassium balance to account for either the early rise in citric acid excretion following adrenalectomy in Patient E. B. (Fig. 3) or the prompt and persistent fall in citric acid excretion in Patient C. V. (Fig. 4). In the other studies, potassium excretion varied inversely with citric acid excretion, as has been reported by Evans and associates (3). We have also observed a rise in urinary citric acid during potassium repletion in an adult patient with Fanconi's syndrome. In Patient C.V., however, retention of potassium occurred during a period of subnormal excretion of citric acid. The continued rise in urinary calcium in the post-treatment control period in Patient C.V. was due to concomitant vitamin D therapy, which probably explains the marked increase in urinary citric acid when cortisone was withdrawn.

The pattern of peripheral blood concentrations of carbohydrate metabolites in Cushing's syndrome was previously interpreted to indicate that 17-hydroxycorticosteroids inhibit the conversion of pyruvic acid to acetyl Co-A (6). The present data further suggest that such a decrease in the formation of acetyl Co-A might result in decreased production of citric acid itself formed by the coupling of acetyl Co-A with oxaloacetic acid.

The 17-hydroxycorticosteroids may in this manner produce a net decrease in the entrance of 2-carbon fragments into the common metabolic pool.

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EFFECT OF FLUORINE ON THYROIDAL IODINE METABOLISM IN HYPERTHYROIDISM

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ABSTRACT

Prolonged administration of a daily dose of 5–10 mg. of fluoride to patients with hyperthyroidism may cause clinical improvement together with a significant fall in the level of plasma protein-bound iodine and a reduction in the basal metabolic rate. Studies with radioactive fluorine failed to demonstrate any important accumulation of fluorine within the thyroid *in vivo*. Thyroidal, blood and urinary radioiodine studies suggest that fluorine inhibits the thyroid iodide-concentrating mechanism. Fluorine does not impair the capacity of the gland to synthesize thyroid hormone when there is an abundance of iodide in the blood. However, inhibition of the thyroidal concentrating capacity when the total iodide pool is low will impose a critical limitation of hormonal synthesis, and may explain the therapeutic effect.

THE widespread use of fluoride to reduce dental caries has aroused considerable controversy because of the toxicity of this halogen. Under the conditions of relative iodine deficiency of Central Europe, a possible thyroid-inhibiting action of fluorine has elicited the interest of physicians and public health officers. Previous studies on animals and human beings did not demonstrate any significant and reproducible alterations of thyroidal function due to small doses of fluorine which did not exceed a daily intake of 2.0 mg (approximately the doses used for prophylaxis of dental caries). In man, however, administration of larger doses over a longer period of time caused a noticeable reduction of several parameters of thyroidal biologic activity (1–5).

Several authors postulated that goitrous states may be attributed to fluorine intake and, conversely, that this element can be utilized in the treatment of hyperthyroidism (6–8). Other investigators, however, could not reproduce these definite changes in the thyroid, and thus the thyreostatic activity of fluorine is still questioned. Since the hyperfunctioning thyroid is a more sensitive structure than the normal gland, we studied the effect of fluorine on patients with hyperthyroidism. Few clinical studies

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are concerned with physiologic and toxic effects of fluorine over long periods (9). Therefore this investigation was limited to intelligent and reliable patients in whom the course of the disease as well as the intake of prescribed doses and the possible appearance of toxic manifestations could be carefully controlled.

METHODS

Fluorine (in the form of sodium fluoride) was given, orally for the most part, three times daily. Patients who received it intravenously were given a single injection daily. The doses recorded here express the quantity of fluorine $(F)^-$ in ionized form. Despite the relatively large amounts administered (up to 20 mg. of F^- for one injection), neither immediate nor delayed toxic manifestations were observed. Patients with severe hyperthyroidism who were not sufficiently improved after fluorine therapy could not be observed for more than six weeks.

In 15 patients, the basal metabolic rate (BMR) and the level of plasma protein-bound iodine (PBI) were determined before and after the administration of 2–10 mg. of F^- over a period ranging from 20 to 245 days. In 10 patients, the action of fluorine was checked by repeated radioiodine tests (10). The initial slope (T_0') of the uptake curve, which equals the uptake rate of the inorganic iodine of the plasma by the thyroid (11, 12), was taken as an indicator of the cumulative, but usually weak inhibiting action of fluorine.

In 4 cases, simultaneous intravenous injections of 20 mg. of F^- and 10–50 μc. of carrier-free I^{131} were given one week after a previous dose of I^{131} alone, to test the possibility of a mass-action effect due to a high level of plasma fluorine. Thyroidal concentration and urinary excretion of radioactive fluorine were studied in 2 cases. F^{18} was obtained by irradiating an aqueous solution of NaF (20 mg. of F^-), as described elsewhere (5).

RESULTS

BMR and PBI

In 6 of the 15 patients studied, the symptoms of hyperthyroidism were relieved and both the BMR and the plasma PBI concentration fell to normal levels. In 9 other cases, fluorine was clinically ineffective, although an improvement in the BMR or PBI level was often observed. The drop of the mean value for plasma PBI from 9.8 to 6.7 μg. per 100 ml. and of the mean value for the BMR from +37 to +27 per cent indicated definite inhibition of thyroid hyperfunction. In 9 patients the fall of the PBI level was significantly greater than twice the standard deviation of an isolated determination as checked by duplicate measurements. The BMR decreased significantly in 7 cases.

I^{131} tests

Repeated radioiodine tests on 6 female and 4 male patients (Table 1) showed the following:

1) An inhibitory effect of fluorine on the initial thyroidal I^{131} uptake was noted in 7 of 10 patients. In 4 of them (Z. M., L. S., K. A. and N. R.)

TABLE 1. INITIAL THYROIDAL UPTAKE RATE (T_0'), MAXIMUM THYROIDAL UPTAKE, PLASMA PBI CONCENTRATION, AND BMR IN 10 PATIENTS WITH HYPERTHYROIDISM BEFORE, AND AT DIFFERENT STAGES DURING ADMINISTRATION OF FLUORINE

Patient, sex & age (yrs.)	Fluoride therapy	Thyroidal I^{131} tests		Plasma PBI (μg./100 ml.)	BMR (%)
		T_0' (% dose/hr.)	Max. upt. (% dose)		
O.T., ♂, 37	control	87	85	14.9	+77
	NaF, 10 mg. q.d. orally, 48 d.	124	88	12.7	+48
P.A., ♂, 41	control	165		9.6	+25
	NaF, 5 mg. q.d. orally, 54 d.	85		8.2	+25
	NaF, 5 mg. q.d. orally, 150 d.	97		7.0	+19
H.H., ♂, 57	control	82	80	8.1	+65
	NaF, 5 mg. q.d. orally, 58 d.	120	87	9.2	+67
	NaF, 5 mg. q.d. orally, 37 d.	106	86	6.8	+60
G.W., ♂, 47	control	53	70′	10.7	+35
	NaF, 2 mg. q.d. intraven., 20 d.			6.5	+36
	NaF, 5 mg. q.d. orally, 49 d.	48		8.0	+22
	NaF, 5 mg. q.d. orally, 38 d.	44		8.0	+28
	NaF, 5 mg. q.d. orally, 160 d.	41	74	6.6	+28
V.M., ♀, 48	control	200	84	7.1	+55
	NaF, 2 mg. q.d. intraven., 20 d.	205	78	7.8	+63
	NaF, 4 mg. q.d. intraven., 28 d.	135		7.6	+71
L.S., ♀, 58	control	45		6.2	+23
	NaF, 2 mg. q.d. intraven., 26 d.	33		5.8	+29
	NaF, 5 mg. q.d. orally, 50 d.	23			+11
	no treatment, 140 d.	30		9.4	+21
E.R., ♀, 27	control	44		14.2	+69
	NaF, 6 mg. q.d. orally, 90 d.	48		14.2	*
Z.M., ♀, 57	control	58		6.9	+18
	NaF, 4 mg. q.d. intraven., 22 d.	57		5.9	+14
	NaF, 5 mg. q.d. orally, 62 d.	31			
	no treatment, 28 d.	28		6.6	+ 3
	NaF, 6 mg. q.d. orally, 100 d.			5.0	− 5
	NaF, 6 mg. q.d. orally, 33 d.	19		4.0	− 5
K.A., ♀, 54	control	70	81	7.4	+28
	NaF, 6 mg. q.d. orally, 119 d.	34	77	3.7	+ 9
	NaF, 6 mg. +I^-, 120 μg. q.d. orally, 51 d.	10	50	7.7	+26
	NaF, 6 mg. q.d. orally, 70 d.			5.7	+ 3
N.R., ♀, 39	control	97	89	11.3	+39
	NaF, 6 mg. q.d. orally, 48 d.	61	91	6.1	+20
	NaF, 6 mg. q.d. orally, 20 d.			5.3	
	NaF, 6 mg. +I^-, 120 μg. q.d. orally, 50 d.	11		8.7	+ 4
	no treatment, 120 d.	250		12.6	+54
	ClO_4, 1200 mg. q.d. orally, 28 d.	3		4.6	+18

* Not determined because patient was pregnant.

the value of T_0' was reduced to the normal range (Fig. 1) by administration of fluorine over a period of 67 to 245 days. Though not as sensitive a test as the I^{131} uptake rate, the maximum uptake decreased, and thyroidal activity during the first week was also modified (Fig. 2).

2) A definite effect of fluorine usually appeared only after 40–50 days. In 3 patients with moderate or severe hyperthyroidism (P. A., G. W. and V. M.) inhibition of the thyroidal uptake of inorganic iodine resulted in

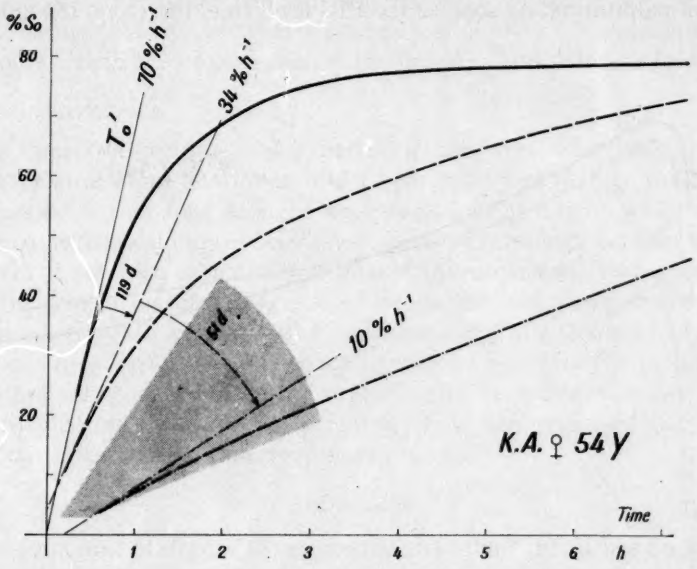

FIG. 1. Variation of the initial thyroidal uptake rate during administration of fluorine in a patient with moderate hyperthyroidism. Ordinate: per cent of the injected dose. Abscissa: time in hours. Dotted sector: zone of the normal values for T_0'. Solid line: control test. Broken line: first test after fluorine administration. Dots and dashes: second test after fluorine administration.

no significant improvement of the clinical picture, and therapy was considered ineffective.

3) In 2 patients (L. S. and N. R.) who showed clinical improvement with a corresponding fall of T_0' to the normal level, interruption of fluorine intake was followed by a relapse of the hyperthyroidism, associated with a rise in the initial slope of the thyroidal iodine uptake.

4) In 2 patients (K. A. and N. R.) with moderate thyroid hyperfunction in whom T_0' was evidently lowered by fluoride therapy, a minimum dosage of inorganic iodine (100–120 μg. of I^- daily) was added to the fluorine

intake. Patient K. A. (recurrence of hyperthyroidism after subtotal thyroidectomy) was clinically cured by fluorine therapy over a period of four months, though a small nodular goiter developed. Administration of iodine caused such a spectacular relapse that it had to be stopped after six weeks. The goiter, which during iodine therapy was no longer palpable, reappeared when hyperthyroidism was controlled by means of fluorine alone. In Patient N. R., the addition of iodine did not alter the clinical effect of fluorine therapy to the same extent. However, there was a recurrence of palpitation. As soon as the PBI level rose, therefore, the adminis-

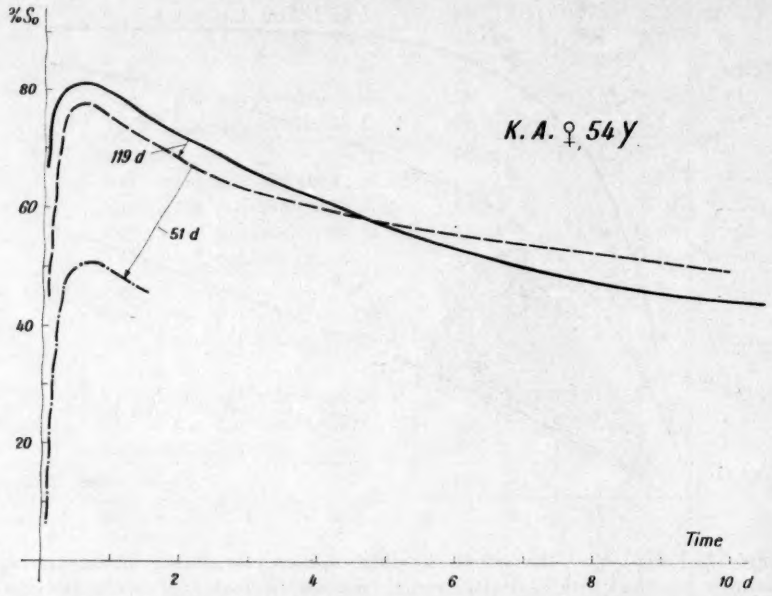

FIG. 2. Variation of the thyroidal uptake curve in the same patient. Ordinate: per cent of the injected dose. Abscissa: time in days.

tration of iodine was discontinued. The patient progressed satisfactorily with fluorine alone for about two months; then she escaped our observation. When seen four months later, she again presented the signs of severe hyperthyroidism. Treatment with sodium perchlorate quickly brought the disease under control.

I^{131}-NaF tests

No inhibition of the rate of thyroidal iodine uptake was observed when fluoride was injected in excess simultaneously with carrier-free radioiodine. On the contrary, the sharp rise of the plasma fluoride level resulted

in a slight increase of the thyroidal uptake rate of inorganic iodine in the 4 cases tested.

F^{18} tests

Radiofluorine was injected into a subject with a normal thyroidal iodine uptake and into a patient with severe hyperthyroidism. In both cases comparison of the radioactivity in the region of the thyroid with that in the region of the precordium did not show any appreciable thyroidal uptake (*i.e.*, less than 1 per cent per hour) of F^{18}, irrespective of the uptake rate of iodine. The rate of urinary excretion of fluorine (29 and 49 per cent per hour) was in the upper range of the urinary excretion rate for iodine.

Clinical observations

The signs and symptoms of hyperthyroidism were completely relieved by administration of fluorine in 6 of 15 patients; tachycardia and tremor disappeared within four to eight weeks and loss of weight was stopped. The greatest clinical improvement was observed in women between 40 and 60 years of age with a moderate degree of thyrotoxicosis. Young patients with the classic picture of Graves' disease did not respond at all to fluorine therapy. In all cases but 1, enlargement of the thyroid was inconspicuous. In a case of severe hyperthyroidism associated with bilateral exophthalmos, congestion of the eyeballs and chemosis were not benefited by fluorine therapy; the proptosis, however, regressed. No other metabolic effects of fluorine were observed.

DISCUSSION

Baumann and Metzger (13) suggested that the thyroid has an affinity not only for iodine, but also for other members of the seventh periodic group of elements. Several studies utilizing fluorine (14), chlorine (15), bromine (16), astatine (17, 18), manganese (19), technetium (20) and rhenium (21) have consistently demonstrated the ability of the thyroid to concentrate these elements in a ratio of greater magnitude than that measured in other tissues of the body. However recent experiments using radiofluorine in rats (22) have not corroborated the earlier chemical findings of Chang *et al.* (14). In our studies with radioactive fluorine, no appreciable uptake of F^{18} by the thyroid could be detected either in the normal or in the hyperactive gland. The significance of this statement is limited to the accuracy of the measurement, since 1% h^{-1} of the injected fluorine amounts to 200 μg. per hour, a considerable quantity when compared to the 2–10 μg. of iodine hourly taken up by the thyroid. The results of these studies must therefore be confirmed by studies using carrier-free radiofluorine.

Apart from the possibility of direct uptake of fluorine by the thyroid, another interesting hypothesis is that fluorine could have a mass-action effect on the uptake of iodine. Experiments with simultaneous intraperitoneal injections of radioiodine and fluorine in rats showed no significant difference in the uptake gradient during the first ten minutes (23). Similarly, in our studies, the sharp rise of the plasma fluorine level did not impair the thyroidal uptake of iodine. Under normal conditions the plasma fluorine level is about four times higher than the iodine level and therefore the action of fluorine upon thyroid physiology is not likely to be the result of simple competition between the halogens for receptor sites within the gland.

Although no toxic action of low fluorine intake upon renal function has been reported (24, 25), it could be assumed that an increase in the urinary excretion rate of iodine would cause a secondary reduction of thyroidal uptake. In 2 cases we measured the initial urinary excretion rate of iodine before and after administration of NaF. In 4 cases the urinary excretion rate was calculated from the values of T_0' and maximum thyroidal uptake (10). No consistent deviation was found.

Another tentative explanation of the depression of the thyroidal iodine-concentrating mechanism is primary inhibition of the synthesis of thyroid hormone, together with a secondary fall of the uptake of inorganic iodine. Thyroidal uptake studies prolonged for seven to ten days, and repeated direct measurements of the level of plasma protein-bound radioiodine before and after fluorine therapy, yield no evidence of such a mechanism.

The only evidence concerning the mode of action of fluorine upon thyroidal function relates to the direct reduction of the uptake rate of inorganic iodine. This interpretation receives further support from 1) the appearance of a goiter, 2) clinical improvement after fluoride therapy, and 3) recurrence of hyperthyroidism when iodine intake was simultaneously increased. Apparently, the inhibition of the iodine-concentrating mechanism is not complete enough in all patients to induce a sufficient degree of iodine deficiency within the gland. Godley and Stanbury (26) observed that when patients were prepared for operation with perchlorate there was a tendency toward relapse when iodine was added during the final stages in order to reduce the vascularity of the goiter. Fletcher *et al.* (27) described an inhibitory action of perchlorate, thiocyanate, iodide and nitrate upon the I^{131} concentration in slices of the salivary gland of the mouse. They found later (personal communication) that the fluoride ion at a concentration of 10^{-3} M depresses the iodide accumulation to about 70–80 per cent of the control value without concomitant depression of the oxygen consumption of the cells. With higher concentrations of fluoride, both oxygen consumption and iodide uptake are depressed. It is

therefore not surprising that the action of fluorine upon the thyroidal uptake of iodide in man has been found weak, inconstant and delayed and that hyperthyroidism recurs when blood iodide levels are sufficiently high to raise the concentration within the gland to the level previously reached by the gland's iodide-concentrating mechanism alone.

The main objective of the present study was not to determine whether fluoride is effective, safe and suitable for routine use in the medical treatment of hyperthyroidism. For instance, with the same biologic mechanism of action, perchlorate is a more active drug and its use does not raise the question of possible accumulation in the body. Our aim was to elucidate the inhibitory effect of chronic administration of fluoride upon thyroid function in cases of hyperthyroidism. It was demonstrated that such an action appears only occasionally among persons subjected to massive doses of this substance.

Acknowledgment

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EFFECTS OF HYPO- AND HYPERTHYROIDISM ON THE FILTERABILITY OF SERUM MAGNESIUM

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ABSTRACT

The concentrations of total and ultrafilterable magnesium were measured in 16 normal subjects, 18 patients with thyrotoxicosis, and 10 with primary or secondary myxedema. In a small number of patients the measurements were repeated after correction of thyroidal disorders. Anaerobic ultrafiltration of the serum was accomplished by using the Lavietes ultrafiltration unit. The total serum magnesium level of the normal subjects averaged 2.13 mg. per 100 ml., and the mean concentration of ultrafilterable magnesium was 1.58 mg. per 100 ml. The latter represented 74.2 per cent (mean value) of the total magnesium content of the serum. The concentrations of total and ultrafilterable magnesium in the serum of the patients with thyrotoxicosis and myxedema did not differ significantly from those of the normal subjects.

BOTH magnesium and calcium, the major divalent cations in the body, form weakly dissociated complexes with the plasma proteins (1–3). It is generally believed that the protein-bound or nondiffusible form of these ions is of little physiologic significance in human subjects (2, 3), and that the homeostatic regulation of the serum levels of magnesium and calcium concerns only the ionized or diffusible form of these electrolytes (1–3). The characteristics of protein-binding for both of these ions are very similar, and the dissociation constants may actually be the same. This may be expressed as follows:

$$\frac{(Ca^{++})(Prot^{=})}{(Ca\ Prot)} = K = \frac{(Mg^{++})(Prot^{=})}{(Mg\ Prot)} \qquad (1,2)$$

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With regard to calcium, it has been shown that pathologic states associated with hypo- or hypercalcemia do not alter this fundamental relationship (3).

TABLE 1. TOTAL AND ULTRAFILTERABLE MAGNESIUM IN SERUM OF NORMAL SUBJECT

Subject No.	Sex	Magnesium		
		Total (mg./100 ml.)	Filterable (mg./100 ml.)	Filterable (%)
1	M	1.75	1.40	80.0
2	M	2.20	1.60	72.7
3	M	2.34	1.74	74.4
4	F	2.49	1.92	77.2
5	M	2.14	1.68	78.5
6	M	2.20	1.40	63.6
7	M	2.48	1.79	72.2
8	M	2.00	1.72	86.0
9	M	2.28	1.69	74.2
10	F	2.28	1.75	76.8
11	M	2.11	1.58	75.0
12	M	1.86	1.35	72.5
13	M	1.85	1.00	54.0
14	F	1.97	1.47	74.6
15	M	1.95	1.60	82.0
16	M	2.21	1.61	73.0
Mean+S.D.		2.13±0.22	1.58±0.22	74.2±7.3

Dine and Lavietes (4) and Soffer and his associates (5, 6) reported that the binding of magnesium to plasma proteins was altered in thyroid disease, the magnitude of the binding being decreased in myxedema and increased in thyrotoxicosis. Their findings have not been confirmed by subsequent investigators (7–9). Because of these conflicting results and the recent demonstration of the important basic interrelationship between thyroid and magnesium metabolism (10, 11), the present study was undertaken in order to re-examine the effect of thyroid disease on the concentrations of total and ultrafilterable magnesium in serum.

METHODS AND PROCEDURE

The concentration of total and ultrafilterable magnesium in serum was measured in 16 normal subjects, 18 patients with thyrotoxicosis, and 10 with primary or secondary myxedema. In a small number of patients, the measurements were repeated after correction of the thyroidal disorder. While the subjects were in the fasting state, venous blood was collected in an oiled syringe and transferred under oil to centrifuge tubes.

TABLE 2. TOTAL AND ULTRAFILTERABLE MAGNESIUM OF SERUM IN THYROTOXICOSIS

Patient No.	Sex	Serum BEI or PBI (μg./100 ml.)	Serum magnesium		
			Total (mg./100 ml.)	Filterable (mg./100 ml.)	Filterable (%)
1	F	15.7	2.04	1.46	71.6
2	F	11.0	2.04	1.68	82.4
3	F	10.8	2.15	1.61	75.0
4	F	11.7	1.88	1.50	79.8
5	F	8.3	2.49	1.89	75.9
6	M	9.1	2.08	1.69	81.2
7	F	11.0	1.96	1.34	69.0
8	F	7.5	2.17	1.22	56.1
9	F	14.9	2.07	1.82	88.0
10	F	8.7	2.91	2.18	75.0
11	M	9.2	2.28	1.50	65.8
12	F	14.0	2.20	1.20	54.5
13	M	13.0	1.90	1.30	68.4
14	M	10.0	2.00	1.60	80.0
15	M	15.0	1.85	1.40	75.6
16	F	14.0	1.70	1.40	82.3
17	M	13.0	2.20	1.70	77.2
18	M	9.5	2.00	1.60	80.0
Mean+S.D.			2.08±0.23	1.56±0.19	74.3±8.9

Following centrifugation the serum was either added directly to the anaerobic ultrafiltration unit or stored under oil in the frozen state. This storage did not alter the pH of the serum or the diffusibility of the magnesium.

Anaerobic ultrafiltration of the serum was carried out in units designed by Lavietes (12), through a visking membrane, utilizing 35 cm. of mercury pressure. Ultrafiltrate was collected at room temperature for a period of twelve to sixteen hours. The pH of the filtrate did not differ significantly from that of the original sera. The concentration of magnesium in serum and ultrafiltrate was determined by a modification of the titan-yellow method of Young and Gill (13).

RESULTS

The individual and mean values for total serum magnesium and ultrafilterable magnesium for all subjects are presented in Tables 1–3. The mean concentrations of magnesium in the sera of the normal, thyrotoxic and myxedematous subjects were 2.13 mg., 2.08 mg. and 2.30 mg. per 100 ml., respectively, and the mean concentrations of ultrafilterable magnesium were 1.58 mg., 1.56 mg. and 1.74 mg. per 100 ml. When the values for ultrafilterable magnesium in the normal, thyrotoxic and myxedema-

TABLE 3. TOTAL AND ULTRAFILTERABLE MAGNESIUM OF SERUM IN MYXEDEMA

Patient No.	Sex & Diagnosis*	Serum PBI (μg./100 ml.)	Serum magnesium		
			Total (mg./100 ml.)	Filterable (mg./100 ml.)	Filterable (%)
1	M^1	3.0	2.37	1.86	78.5
2	M^1	2.0	2.59	1.95	75.2
3	M^2	1.8	2.17	1.64	75.5
4	M^2	2.2	2.35	1.63	69.3
5	M^2	2.7	2.30	1.44	62.3
6	M^3	2.9	2.65	1.96	73.9
7	M^3	2.3	2.12	1.45	68.3
8	M^3	1.7	2.49	2.00	80.3
9	F^3	1.6	1.75	1.29	73.7
10	M^4	2.3	2.25	2.17	96.5
Mean+S.D.			2.30±0.26	1.74±0.29	75.4±9.01

* 1 = Primary myxedema.
2 = Pituitary myxedema.
3 = Post-I^{131} myxedema.
4 = Postoperative myxedema.

TABLE 4. PAIRED DETERMINATIONS ON THE SAME SUBJECT BEFORE AND AFTER THERAPY

Sex of subject	Diagnosis	Serum magnesium		
		Total (mg./100 ml.)	Filterable (mg./100 ml.)	Filterable (%)
F	1) Thyrotoxicosis	1.70	1.40	82.3
	2) Post-I^{131} myxedema	1.75	1.29	73.7
F	1) Thyrotoxicosis	2.20	1.20	54.5
	2) Euthyroid with propylthiouracil	2.30	1.20	52.1
	3) Euthyroid after thyroidectomy	2.80	1.70	60.7
M	1) Thyrotoxicosis	1.80	1.40	77.8
	2) Euthyroid after I^{131}	2.00	1.30	65.0
M	1) Thyrotoxicosis	2.28	1.50	65.8
	2) Post-I^{131} myxedema	2.12	1.45	68.4

tous subjects were expressed as a percentage of the total, they were 74.2, 74.3 and 75.4 per cent, respectively.

In the present study, therefore, hypo- and hyperthyroidism did not significantly alter the content of total or ultrafilterable magnesium in serum, compared with the values observed in the normal subjects ($P > > .05$). Furthermore, in any given patient, the conversion from thyrotoxicosis to euthyroidism or myxedema was not associated with a consistent change in the partition of magnesium in the serum (Table 4). One myxedematous patient (No. 10, Table 3) had the highest level of ultrafilterable magnesium observed (96.5 per cent). This man had had a total thyroidectomy for giant-cell thyroiditis. Analysis of his plasma disclosed a normal amount and electrophoretic distribution of proteins.

DISCUSSION

The results of the present study confirm those of previous investigators who have been unable to demonstrate a consistent effect of hypo- and hyperthyroidism on the filterability of serum magnesium (7–9). Approximately 75 per cent of the total serum magnesium was ultrafilterable regardless of the level of thyroidal function. This is slightly higher than the 65 to 70 per cent observed in other studies. However, in the present investigation great care was taken to preserve the anaerobic character of the serum and the changes in pH were minimal. The steps necessary to maintain a constant pH were not observed in some of the other investigations (7–9). Howard and his associates have shown that an increase in the pH of serum will decrease the ultrafilterability of both calcium and magnesium (3).

These results do not confirm the earlier observations of Soffer *et al.* (5, 6) and Dine and Lavietes (4) that hypo- and hyperthyroidism alter the binding of magnesium to plasma proteins. An examination of their clinical and laboratory data does not disclose an adequate explanation for these differences. It is of interest, however, that the normal subjects in Soffer's study had very low levels of protein-bound magnesium (3 to 20 per cent), whereas his thyrotoxic subjects had levels ranging from 21 to 61 per cent.

In spite of the negative results of the present investigation, there is little doubt that the metabolic effects of thyroxine are intimately influenced by magnesium ion. Administration of thyroxine or triiodothyronine to human subjects causes an immediate negative balance of magnesium (10). Typical magnesium deficiency with poor growth, lowered concentration of serum magnesium and deficiency symptoms can be readily produced by the continued administration of thyroxine (11). It is evident that thyroxine therapy increases the body's requirement for magnesium.

It seems reasonable to conclude that although thyroid hormone can exert a pronounced effect on the metabolism of magnesium, it does not

alter the fundamental mass-action interrelationship between the plasma proteins and magnesium ion (1, 2).

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Letters to the Editor

COLLABORATIVE ASSAYS OF TWO URINARY PREPARATIONS OF HUMAN PITUITARY GONADOTROPIN

To the Editor:

Methods depending on kaolin adsorption and elution followed by acetone precipitation are being widely used to extract gonadotropin from the urine of nonpregnant subjects (1–5). The presently available evidence supports the view that none of the factors necessary for interstitial-cell repair, for spermatogenesis or for secretion of sex hormones by the gonads are eliminated or destroyed by these procedures (5–7). However, the bioassay of extracts prepared by these kaolin-acetone methods has, until now, not been entirely satisfactory because of the lack of a suitable reference standard.

In the absence of a standard preparation, results of assays can be expressed only in terms of various "animal units," and this has made it very difficult to compare results obtained in one laboratory with those obtained in another. Furthermore, assays conducted without a standard are subject to many sources of variation, the magnitude of which is not known but which must be considerably greater than in assays in which a standard preparation is used.

Recently, 2 reference standards have been prepared by kaolin-acetone methods; one is from the urine of menopausal and postmenopausal women, hereafter termed *HMG-20A* (8), and the other is from the urine of normal males, hereafter termed *AMW* (9). Although the kaolin-acetone methods used to prepare these 2 local standards are very similar, they are not identical and, therefore, it is possible that the 2 preparations may differ both physically and chemically. One milligram of HMG-20A represents the gonadotropic activity contained in approximately 10 ml. of the original urine, whereas 1 mg. of AMW represents the activity of approximately 50 ml. of the original urine. It should be emphasized that the methods used to prepare the 2 local standards are very similar to those routinely employed in the respective laboratories for the extraction of gonadotropin from urine for clinical purposes.

The purpose of the present investigation was to undertake a collaborative assay of AMW in terms of HMG-20A in order to determine whether such local standards could serve as forerunners of a more widely used standard material. A study or a series of studies of this sort generally has been conducted in order to provide advance information bearing on the question as to whether the establishment of an international standard would serve a useful purpose.

METHOD AND RESULTS

The collaborative assay was performed in 6 different centers. In the present paper, the results reported are those obtained by assay methods that, so far, have been used in more than one laboratory. The methods were 3 in number and were as follows: (a) the uterine weight test in intact immature mice, (b) the ovarian weight test in intact immature rats, and (c) the ventral prostatic weight test in hypophysectomized immature rats.

These methods have been widely used for many years and require no further description of technical details, which vary in minor ways among the collaborating laboratories.

The results of the collaborative assay, arranged according to the assay procedure, are shown in Table 1. The statistical methods[1] used for calculating the relevant parameters have been fully described elsewhere (10, 11). All assays were reasonably precise and satisfied the recognized criteria of validity.

The mouse-uterus test was used in 4 different laboratories; 7 assays were performed. The unweighted mean relative potency of AMW in terms of HMG-20A was 0.62. A high degree of internal uniformity was observed and there was complete overlap of confidence limits, that is, the upper confidence limit of all 7 assays was not less than the lower confidence limit in any of the assays. The test employing prostatic weight in hypophysectomized rats was used in 2 different laboratories; 3 assays were performed. The unweighted mean relative potency was 0.42, and there was complete overlap of confidence limits. The ovarian weight test was used in 2 different laboratories; 4 assays were performed. The unweighted mean relative potency was 0.90. Two of the upper confidence limits (Edinburgh) were less than the lower confidence limit of 1 of the assays performed in Rochester. Examination of Table 1 as a whole indicates that only partial overlap of confidence limits occurs among the 3 methods of assay.

The mean relative potency calculated from all 14 assays was 0.64, and the span (highest figure divided by lowest) was 3.6. If it be assumed that mean confidence limits of 0.46 to 1.15 represent a crude approximation of

[1] We have been privileged to consult Prof. J. Berkson (Rochester), Prof. J. Gaddum (Edinburgh) and Prof. A. Linder (Geneva) with respect to statistical considerations.

TABLE 1. COLLABORATIVE ASSAY OF AMW IN TERMS OF HMG-20A*

End point	Place	Design	N	λ	R.P.	Confidence limits†
Uterine weight, intact mice	Edinburgh	2 & 2	14	0.14	0.64	(0.43–1.69)
	Edinburgh	2 & 2	16	0.11	0.58	(0.44–0.82)
	Geneva	2 & 2	14	0.21	0.75	(0.33–3.16)
	Geneva	2 & 2	18	0.09	0.71	(0.56–0.88)
	Geneva	2 & 2	19	0.21	0.63	(0.17–1.50)
	Stockholm	2 & 2	40	0.13	0.57	(0.44–0.69)
	Worcester	3 & 3	57	0.18	0.51	(0.39–0.67)
Ventral-prostate weight, hypophysectomized rats	Boston	3 & 3	27	0.12	0.34	(0.27–0.43)
	Edinburgh	2 & 2	17	0.08	0.49	(0.41–0.54)
	Edinburgh	2 & 2	21	0.06	0.46	(0.39–0.51)
Ovarian weight, intact rats	Edinburgh	2 & 2	16	0.11	0.63	(0.37–0.99)
	Edinburgh	2 & 2	16	0.15	0.75	(0.40–0.96)
	Rochester	3 & 3	26	0.08	1.22	(1.05–1.40)
	Rochester	2 & 2	16	0.15	1.12	(0.74–1.75)

* N = Total number of animals; λ = index of precision; R.P. = relative potency, or units per milligram, 1 unit being the activity contained in 1.0 mg. of HMG-20A.

† P = 0.95.

the variation to be expected from the intra-assay error, then 2 values, namely 0.34 (Boston) and 1.22 (Rochester), appear to be distinctly outside the expected range of variation. Indeed, analysis of variance of the 14 values for relative potency showed that these estimates differed more than would have been anticipated from the calculated pooled intra-assay variation. The differences appeared to be due not so much to variation in replication of any one method by any one laboratory or to variation between laboratories using the same assay method, but rather to additional sources of random experimental variation contributed largely by the various methods themselves as used in the different centers. Differences in estimated potency of this magnitude (0.34 to 1.22) are unlikely to result by chance as judged by the internal evidence of the assay. They probably could arise from such factors as inter-assay error (12), from physical, chemical and biologic differences in the 2 standards, and from difficulties associated with the prediction of the error of an assay by internal evidence alone. It should be borne in mind that, although the mathematical model underlying the currently used fiducial limits may not be adequate under all experimental conditions, nevertheless, the calculation of these limits generally will provide a very useful estimate of the variation to be expected.

DISCUSSION

Despite some uncertainties, the data presented in this paper provide preliminary evidence that the establishment of an international standard for human pituitary gonadotropin would serve a useful purpose. This statement appears justified for the following reasons:

A. Validity tests were satisfactory in all the assays performed. In particular, there was no significant deviation from parallelism in any assay.

B. The variation between replicates, and the magnitude of the interlaboratory errors were relatively small.

C. The standard would provide the basis for conducting proper clinical assays or for carrying out the necessary research in order to determine how to conduct proper clinical assays.

The opinion expressed in this paper is at variance with that of Hamburger and Johnsen (13) and Johnsen (14), who stated that the establishment of an international standard for human pituitary gonadotropin cannot be recommended. The basis for their conclusion was the claim that the gonadotropic material excreted by normal men differed qualitatively from that excreted by postmenopausal women. Specifically, it was stated that absolutely and relatively more follicle-stimulating hormone (FSH) was present in the urine of postmenopausal women than in the urine of men, and that the luteinizing potency of male urine was two to three times greater than that of postmenopausal urine.

That the gonadotropic activity of male urine may differ qualitatively from that of postmenopausal urine is of itself an important problem. However, in our opinion, the presence or absence of qualitative differences does not necessarily bear a direct relationship to the establishment of an international standard. The data presented in this paper do not yield definite information on the question of qualitative differences; this may be due to the fact that the 3 assay methods selected are not capable of discriminating between such differing proportions of FSH and interstitial cell-stimulating hormone (ICSH) activities as may be present in the urine of men and of postmenopausal women.

It was considered desirable to apply Gaddum's (15) index of discrimination to the data shown in Table 1. This index is calculated by dividing the mean potency ratio in one test by the corresponding figure in another. It is close to unity when 2 substances possessing identical biologic activity are assayed, and when inert or other material present in the 2 preparations does not seriously interfere with the estimate of relative potency. The mean index of discrimination for the 3 assay methods used is shown in Table 2. It will be noted that the values were not greatly divergent from unity.

The index of discrimination has proved of considerable value as a means

TABLE 2. MEAN INDEX OF DISCRIMINATION FOR 3 ASSAY METHODS FOR GONADOTROPIN

Combination of assays	Mean index of discrimination
Ovary (rat)/uterus (mouse)	1.45
Ovary (rat)/prostate (rat)	2.14
Uterus (mouse)/prostate (rat)	1.47

of comparing the potency of various pharmacologic agents. However, a definite opinion as to its worth in an investigation of the type reported here cannot be given at present. In particular, it remains uncertain as to how much the index must deviate from unity before the presence of a qualitative difference is established.

The index of discrimination also can be applied to the data reported by Hamburger and Johnsen (13), who compared the activity of male and of postmenopausal female urines using the mouse-uterus test, the corpus-luteum test in mice, and the prostate test in hypophysectomized rats. They stated that, when doses of extracts equal in potency by the mouse-uterus test were assayed by the other 2 methods, the potency of the extract from male urine was two to three times that of the extract from postmenopausal urine by the corpus-luteum test and that the corresponding figure for the prostate test in hypophysectomized rats was 2.1. In effect, these values represent single estimations of the index of discrimination; it will be noted that they are of the same order of magnitude as those reported here.

Neither the data of Hamburger and Johnsen nor the results in the present paper have provided definite information as to whether male gonadotropin differs qualitatively from postmenopausal gonadotropin. However, it is our opinion that this unsettled question should not delay and does not diminish the need for the establishment of an international standard that, by virtue of its existence, would be of considerable importance in solving this very problem.

CONCLUSIONS

1. The establishment of an international standard for human pituitary gonadotropin in urine is strongly recommended. Such a standard would be of great value in research work, making it possible to compare assay results obtained by different methods and under varying experimental conditions.

2. The possibility that such a standard might contain not a single substance but 2 or more biologically active substances is not an argument against its establishment.

3. Further experimental data are required regarding the possible limitations involved in the prediction of the inter-assay variation from the internal error of the assay.

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ANDROGENIC PATTERN OF SERUM LIPIDS AND LIPOPROTEINS IN RESPONSE TO CHORIONIC GONADOTROPIN IN MALE HYPOGONADISM*

To the Editor:

The intramuscular administration of chorionic gonadotropic hormone (CGH) to boys and to eunuchoidal males in whom the testes are capable of responding leads to testicular maturation, virilization and increased urinary excretion of 17-ketosteroids (1–3). We have studied the effects of CGH on serum lipids and lipoproteins in such subjects, and have compared the effects with those observed following the administration of androgens and estrogens to other subjects (4).

SUBJECTS AND METHODS

Seven young hypogonadal males, otherwise in general good health, were studied. Two had panhypopituitarism. The others were between 8 and 18 years of age and manifested incomplete sexual maturity; 4 of them were cryptorchid. Chorionic gonadotropin (A.P.L., Ayerst) in the form of a dry powder was dissolved in distilled water before initial intramuscular injection. A weekly dose of 4000 to 6000 international units was

* This study was supported in part by grants from the National Heart Institute (H-1429 and H-1694) U.S.P.H.S., the Oklahoma State Heart Association, and the Ayerst Laboratories (New York, N. Y.).

TABLE 1. EFFECTS OF ADMINISTRATION OF CGH ON THE PATTERN OF SERUM LIPIDS AND LIPOPROTEINS AND ON URINARY EXCRETION OF 17-KETOSTEROIDS

(lipids and lipoproteins in terms of mg. per 100 ml. ±S.E.)

Subject	Cholesterol	Phospholipid	$-S_{1.21}$ 0–12 ("Alpha")	$-S_{1.21}$ 25–40 ("Beta")	$-S_{1.21}$ 0–12 / 25–40	Urinary 17-KS (mg./24 hrs.)	Therapy
E.S. (U.H. 218310)	156 ±12	266 ±11	230 ±22	179 ±14	1.33 ± .17	2.1 ± .2	No Rx.
	ns*	↓ .02	ns	ns	ns	↑ .01	
Aged 18	138 ± 5	226 ± 6	190 ± 7	172 ± 9	1.12 ± .10	3.7 ± .2	CGH, 2500 I.U. twice weekly
	↑ .001	↑ .001	↑ .001	ns	↑ .01	ns	for 18 wks.
	188 ± 9	272 ± 4	317 ±14	170 ± 7	1.89 ± .14	2.6 ± .5	Stopped Rx.
G.S. (U.H. 177643)	123 ± 3	201 ± 5	159 ± 5	127 ± 5	1.26 ± .06	1.6 ± .4	No Rx
	↓ .001	↓ .001	↓ .01	ns	ns	↑ .001	CGH, 2000 I.U. thrice weekly
Aged 15	102 ± 4	166 ± 7	129 ± 7	119 ± 6	1.10 ± .06	4.5 ± .4	for 18 wks.
	↑ .001	↑ .05	↑ .001	↑ .01	ns	↓ .01	
	142 ± 4	190 ± 6	177 ± 8	153 ± 5	1.16 ± .04	2.8 ± .3	Stopped Rx.

* The third column under each heading is the P value as determined by the *t* test. Arrow indicates direction of change; "ns" means "not significant," *i.e.*, $P>0.05$.

given in two or three injections over a period of five to nineteen weeks. No dietary restrictions were imposed.

The methods used for the determination of serum lipoproteins, cholesterol and phospholipid have been described in detail elsewhere (4). Total neutral 17-ketosteroids were determined by the method of Klendshoj, Feldstein and Sprague (5).

RESULTS

The effects of CGH on the pattern of serum lipids and lipoproteins and on urinary 17-ketosteroid excretion were greatest in the 2 subjects with panhypopituitarism (Table 1). High density—$S_{1.21}$ 0–12 or "alpha" lipoprotein concentrations fell during treatment in all subjects and rose after cessation of treatment. The changes in alpha lipoprotein concentrations were statistically significant in 9 of 15 instances. The mean reduction for the group (35 ± 8 mg. per 100 ml.) was significant at the 0.01 level. The likelihood that this consistency of alpha lipoprotein response was due to chance alone is extremely small ($1/15^2$).

Lower density—$S_{1.21}$ 25–40 or "beta" lipoprotein concentrations usually changed in the same direction as those of alpha lipoprotein, but to a lesser extent. This similarity of response of the major lipoproteins was reflected in decreased levels of cholesterol and phospholipid during treatment and increased levels after treatment, with little change in the cholesterol/phospholipid ratio.

The alpha/beta lipoprotein ratios fell during treatment and rose afterward, but the changes were not as striking as those in alpha lipoprotein because of similar, although less marked, changes in beta lipoprotein. The mean reduction of the alpha/beta ratio for the group was statistically significant ($P < 0.001$), but individual reductions were sometimes not statistically significant.

A significant increase in urinary 17-ketosteroid excretion was noted during treatment in 4 of the 7 subjects.

The relatively small changes in serum lipid and lipoprotein concentration and in urinary 17-ketosteroid excretion noted in some subjects following administration of CGH may be related to testicular immaturity and the presence of small amounts of endogenous gonadotropins.

DISCUSSION

Administration of androgen always results in decreased concentrations of high density alpha lipoprotein, whereas administration of estrogen results in increased concentrations (4). The fact that lower density beta lipoprotein concentrations did not change significantly in these subjects is of interest. When weak androgens, or low doses of more potent androgens, are administered, beta lipoprotein concentrations either may not change significantly or may change in the same direction as the alpha lipoprotein

concentrations (6). It is possible that administration of CGH results in the production of estrogen as well as androgen, or in the transformation of some androgen to estrogen in the liver or other tissue. Increased urinary excretion of estrogen and 17-ketosteroids has been noted following administration of CGH to normal adult men and eunuchoidal subjects by Maddock and Nelson (7) and by Leach and coworkers (8). However, our experience with the concomitant administration of "physiologically equivalent" doses of androgen and estrogen indicates that the serum lipoprotein changes are characteristic of androgen therapy (4).

Therefore, administration of chorionic gonadotropin hormone to hypogonadal males, in whom the testes are capable of responding, elicits a serum lipid and lipoprotein response which may be interpreted as due to androgen, regardless of whether such administration results in the release of androgen alone or a mixture of androgen and estrogen.

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PROLONGED TURNOVER RATE OF HYDROCORTISONE IN THE NEWBORN INFANT*

TO THE EDITOR:

In the course of the study of disappearance rates of 17-hydroxycorticoids from plasma following the intravenous administration of hydrocortisone, it became apparent that the half-life of this steroid, measured as phenylhydrazine-reacting compounds ((1) Method #2), was very much prolonged in the newborn human infant.

It has been shown (2, 3) that the disappearance of hydrocortisone after intravenous infusion into adults is a first-order reaction after the first hour. This has been confirmed in children over 2 years of age and in adults, in this laboratory. It was also found to be true in 2 infants, aged 36 and 48 hours, after the administration of the hemisuccinate of hydrocortisone (equivalent to 25 mg. of the free steroid) by vein within one minute and the collection of specimens of blood at hourly intervals for four hours. However, the disappearance rate was quite slow and it became desirable to study a larger group. For this purpose, it became necessary to limit the volume of blood collected, and therefore only the one-hour and three-hour levels could be determined. In view of the nature of the regression curve, this procedure appeared to be valid. In order to check the reliability of this approach, the half-life was measured in 4 subjects (the 2 infants mentioned, and 2 children over the age of 2 years), using four-hour specimens. The calculation, employing the method of the "best fit" for 4 specimens, was compared with that based on the one-hour and three-hour levels only: the agreement was within 1 to 4 per cent. The volume of plasma employed was generally 2.0 ml. in children and infants, and all levels lay between 72 and 978 micrograms per 100 ml., so that the absorbances were above 0.100 at 410 mμ in all studies. In 4 instances, duplicate specimens of plasma were

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examined by the Porter-Silber reaction, following elution of the hydrocortisone area from paper chromatograms resolved in our system EB6(4); the levels were 78–85 per cent of those determined by the routine technique (1).

Subjects under 2 years of age received the equivalent of 25 mg. of hydrocortisone by vein, as the hemisuccinate; the remainder were given the equivalent of 50 mg. of hydrocortisone. Peterson and associates (3) showed that there is no significant difference in the biologic half-life over a wide range of dosage. Values for the half-life of hydrocortisone at various ages are listed in Table 1. On the first day of life, despite the wide range of

TABLE 1. HALF-LIFE OF ADRENAL STEROIDS ADMINISTERED TO SUBJECTS OF VARIOUS AGES

Number of subjects	Age	Steroid admin'd	Half-life of admin'd steroid (mins.)		
			Mean	S.D.	Range
9	1 day	F	977.4	740	250–2185
2	36–88 hrs.	F			490– 758
11	3– 9 days	F	175	31.7	145– 251
10	1–12 yrs.	F	96.0	17.6	76– 124
8	20–35 yrs.	F	112	14.1	88– 131
2	1 day	Tet-E			34– 48
2	13–14 yrs.	Tet-E			21– 48

values, the disposition of hydrocortisone was considerably slower than in all other age groups; it became more rapid by the third to ninth days, but was still less efficient than in older children, However, the disappearance rate of tetrahydro-E (pregnane-3,alpha,17-alpha,21-triol,11,20-dione) was very rapid in 2 infants and in 2 older children, being comparable to the rate observed in adults (2). The results in adults were similar to those reported by others (2, 3, 5, 6). In children between 1 and 12 years of age the values were somewhat less than those for adults, as in the studies of Done *et al.* (7). In 5 cases (Table 2), the half-life of hydrocortisone on the first day of life was compared with that on the third or fifth day in the same infant. With increasing age, there was a diminution in the time required for the disposition of hydrocortisone during the first week of life.

The conjugates of 17-hydroxycorticoids were also measured in all specimens, following hydrolysis with beta-glucuronidase (liver). These results are not shown in the tables, since the half-life could not be accurately expressed in all age groups. Within the first few days of life, employing only the one-hour and three-hour levels, the biologic half-life of the conjugates

TABLE 2. HALF-LIFE OF HYDROCORTISONE AT 2 AGES IN EACH OF 5 INFANTS

Infant No.	Half-life of hydrocortisone (mins.)		
	Day 1	Day 3	Day 5
1	1755		214
2	1265		290
3	307	148	
4	427	197	
5	318	145	

varied between 30 and 60 minutes in all infants. However, in older children and adults, it was much longer; in fact, within the narrow range of time for the conduct of the investigation, the three-hour level was often higher than the one-hour level. Brown and associates (2) have shown that the peak for conjugated 17-hydroxycorticosteroids in adults occurs later than that for the free steroid and is presumably influenced by the continued contribution from the "free" pool, as well as excretion. During the interval within which the former exceeds the latter, a diminution in blood level may not be observed. However, the short half-life of the conjugates in infancy reflects 2 facts: 1) there is little if any interference with the excretion of conjugated corticoids, and 2) the rate of conversion of hydrocortisone to conjugated steroids is much slower in the early days of life. The latter circumstance is also upheld by the tardy disappearance of free 17-hydroxycorticosteroids (Table 1).

Grodsky *et al.* (8) have reported that the level of glucuronic-acid transferase, described by Isselbacher and Axelrod (9), is low or absent in early life. This would not satisfy the requirements for the long half-life of hydrocortisone in early life. The rapid disappearance of tetrahydro-E observed in this study is not consistent with this thesis. Indeed, it would appear that this particular mechanism is wholly intact during the first days of human life, with respect to hydrocortisone. Peterson and Schmid (10) noted such a defect in a special instance wherein the disposition of tetrahydro-E was delayed. Rather it would appear that relative lack of the enzyme(s) involved in the reduction of ring A of hydrocortisone (11) is to be incriminated during early human life. This has not been proved by our studies.

Migeon *et al.* (12) have shown that the half-life of hydrocortisone during late pregnancy is approximately twice that in control subjects. There is, then, a similarity between the pregnant mother and her newborn infant. Wallace *et al.* (13) reported that, in man, the administration of estrogens delays the disposition of hydrocortisone. Might the maternal estrogens,

transmitted to the infant, account for our findings? Diczfalusy *et al.* (14) have recently shown that in the urine of newborn infants there is a large amount of estriol, which diminishes rapidly to negligible levels by the fifth day. This finding parallels the changes in the disposition of hydrocortisone during early life, which we have described (Table 1).

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OSTEOPOROSIS IN HYPERADRENOCORTICISM

TO THE EDITOR:

Dr. Moldawer's letter (*J. Clin. Endocrinol. & Metab.* **18**: 1028, 1958) concerns a most interesting point and one about which I am aware of no discussion in the literature. It is also an important and practical one, if it should prove true that osteoporosis due to hyperadrenocorticism can be arrested but is not reversible. I suspect that is not so, in the strict sense of the term, *i.e.*, that the porotic bone cannot ever be remodeled and become histologically indistinguishable from normal bone.

It is true that, in Dr. Skeels' recently reported x-ray pictures (*J. Clin. Endocrinol. & Metab.* **18**: 61, 1958) and doubtless also in the Albright case even eleven years after arrest of Cushing's disease, there is a central square in the vertebrae which looks as if the original porotic area remained, surrounded by good, dense, normal-appearing bone, adequate for normal support. Yet it somehow does not seem likely that this occurs in all bones after hyperadrenocorticism has been removed, or that it should be forever impossible for such tissue to be resorbed and remodeled.

When puzzled about bone injury, growth and regeneration, I always go to Dr. Edward A. Park and he usually comes up with the answer. In this instance he was at once reminded of what happens when scurvy heals. In scurvy there is also general skeletal rarefaction (osteoporosis). In the centers of ossification of the head of the tibia or femur (all centers show it), the bone appears rarefied, as do all the other bones. When scurvy heals, these centers grow and the new bone surrounding them appears far more dense, but the mid-portion of the center of ossification remains apparently rarefied, at least as long sometimes as three years. This, to the pediatrician, is known as Rehler's sign and is indicative that scurvy has previously been present. In the other bones (not completely surrounded by cartilage), in which metabolic activity seems to be much greater, the porosis disappears very rapidly.

The parallelism seems clear and I suspect that this is the reason for the

apparent slowness of healing or cure of the porosity in the central vertebral area—namely, the metabolic turnover is very slow. If the new surrounding bone is normally dense and strong, the question becomes of academic interest only.

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HISTOLOGIC EVIDENCE OF MALIGNANCY IN A THYROID GLAND BEARING A "HOT" NODULE

TO THE EDITOR:

We recently found an occult, sclerosing carcinoma in a "hot" thyroid nodule. We are not aware of a previously reported case. Perlmutter and Slater (1) proposed evaluation of the probability of malignancy or benignancy in thyroid nodules on a basis which relies upon the absence of any recorded instance of histologic evidence of malignancy in a hyperfunctioning adenoma (2–8). These circumstances prompt us to report the following case.

A 28-year-old clergyman complained of nervousness, irritability, intolerance of heat, excessive sweating and occasional loose stools that had been noted for two or more years. He had been known to have diabetes mellitus for three years. During that time his need for insulin had increased from 40 to 80 N.P.H. units a day. The diabetes had become less stable, in that he had had a bout of diabetic acidosis and frequent insulin reactions within the previous eighteen months.

He was found to be minimally tremulous, with warm but dry skin. His pulse ranged from 98 to 116 beats per minute, and his blood pressure was 116 mm. Hg, systolic, and 80 mm., diastolic. In the left lobe of the thyroid gland a spherical, firm nodule was palpable, estimated to be 3 by 2 by 2 cm. in size. There was minimal nodularity of the right lobe. The basal metabolic rate was +11 per cent. The value for serum protein-bound iodine was 6.6 micrograms per 100 ml. The uptake of radioiodine in nineteen hours was 11.8 per cent, most of this having collected in the clinically palpable nodule described, as shown in the scintigram (Fig. 1). No other abnormalities were detected.

Clinically, the patient appeared slightly hyperthyroid. After preparation with Lugol's solution, thyroidectomy was performed on March 12, 1957, by Dr. E. S. Judd. The left lobe, the isthmus and a portion of the right lobe were removed. The clinically palpable nodule appeared to be a bosselated area of the left lobe which measured 4.5 by 3 by 1.5 cm. over-all and weighed 13 Gm. There was no evidence of encapsulation and no gross nodularity on the cut surface. Histologically, the parenchyma contained small focal areas of hyperplasia with much normal-appearing thyroid tissue. Deep within this lobe was a tumor (0.5 cm. in diameter) which was a so-called occult, sclerosing adenocarcinoma. The removed portion of the right lobe measured 2 by 1 by 1.5 cm. and weighed

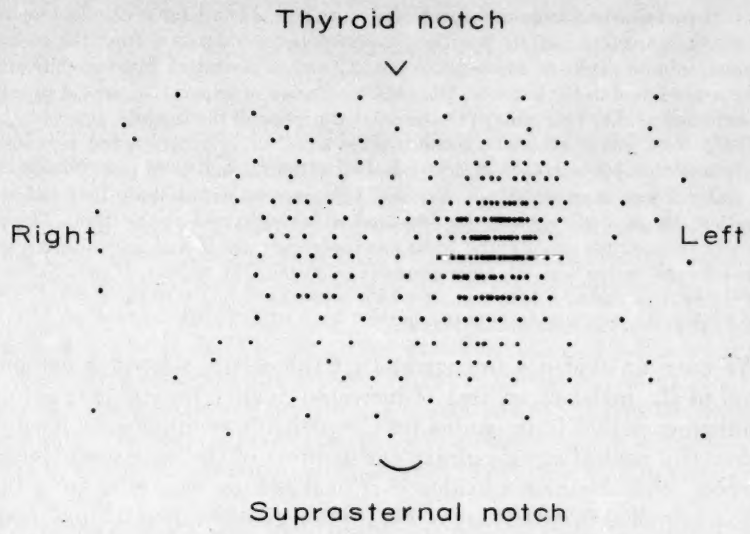

FIG. 1. Scintigram made on February 28, 1957.

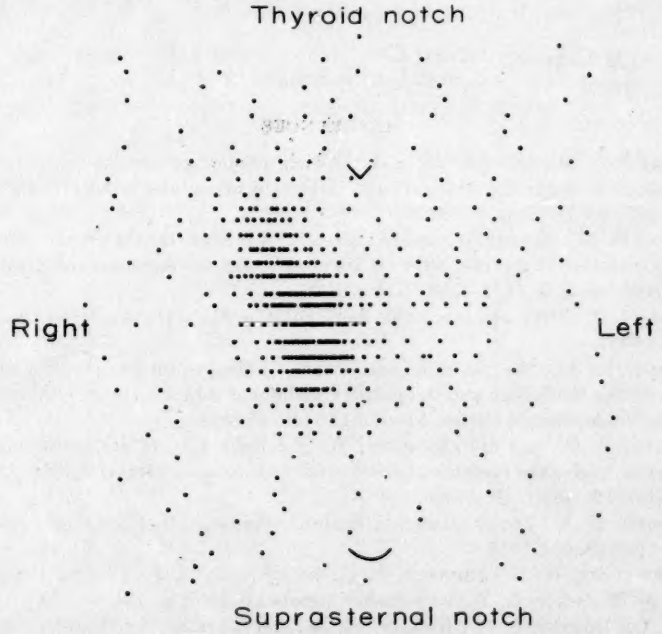

FIG. 2. Scintigram made on September 26, 1957.

4 Gm. It contained no areas of hypertrophy but there was a fetal and colloid adenoma (0.8 cm. in diameter) and, deep within its substance and separate from the adenoma, a second, minute papillary adenocarcinoma (0.2 cm. in diameter), histologically similar to the lesion found in the left lobe. Only the focal areas of hyperplasia served to explain the increased avidity for iodine of the bosselated portion of the left lobe.

The patient was re-examined seven months later. He said he was less nervous and sweaty and that his tolerance of heat was better than it had been prior to operation. His diabetes was more stable and required 35 units less insulin daily than before the operation. By all usual criteria, he appeared to be euthyroid at this time. The pulse rate was 78 beats per minute, the basal metabolic rate was 0, and the value for serum protein-bound iodine was 5.1 micrograms per 100 ml. The uptake of radioiodine was 16.3 per cent in eighteen hours, all of which collected diffusely in the remaining right lobe, as shown in the scintigram (Fig. 2).

We have no evidence to suggest that the occult, sclerosing carcinoma found in the midst of an area of increased avidity for iodine was hyperfunctioning or that it accounted for the patient's symptoms. In itself, it is of doubtful clinical significance. Consideration of this case would suggest, however, that histologic evidence of malignancy can exist in a "hot" nodule as well as in "cold" areas of a thyroid gland bearing a "hot" nodule.

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RENAL CONCENTRATING ABILITY IN THYROTOXICOSIS

To the Editor:

Despite the widespread alterations in many bodily functions induced by thyrotoxicosis, results of conventional tests of renal function in this disease have generally been normal (1–4). Recently, however, Weston and associates (5) reported that 5 severely hyperthyroid women failed to respond with the anticipated antidiuresis and increase in urinary solute concentration, to injections of vasopressin in oil. They suggested that hyperthyroid patients exhibit a greater resistance to vasopressin than might be explained on the basis of either an increased excretion of solute or altered renal hemodynamics. In the present study, renal concentrating ability in response to exogenous vasopressin was measured in 13 thyrotoxic patients. The results (Table 1) do not confirm the hypothesis that a defect in renal concentrating ability is commonly observed in patients with hyperthyroidism.

Ten women and 3 men were studied before the start of medical treatment for outspoken clinical signs and symptoms of hyperthyroidism. The level of serum butanol-extractable iodine was elevated in all patients. A dose of 5 units of vasopressin in oil[1] was injected intramuscularly. The

Table 1. Maximum urinary concentration (U_{max}) in 13 hyperthyroid patients

Patient		Serum BEI (μg./100 ml.)	U_{max} (mOsm/K)
Age (yrs.) & sex			
45	F	10.4	847
52	M	9.1	745
22	F	11.6	950
40	F	10.8	784
29	F	11.0	850
40	F	11.0	800
68	F	12.8	804
24	F	8.9	930
30	F	8.4	896
49	M	14.1	640
60	F	12.6	699
27	M	7.0	729
38	F	12.0	954
Mean 40			818

[1] Pitressin-Tannate in Oil ®, Parke-Davis and Co.

patient was then instructed to void every hour until 4 specimens of urine had been obtained. Fluids were not restricted before or after the test. Urinary osmolality was measured cryoscopically with a Fiske osmometer, and the highest osmolality of any of the 4 urine samples was recorded as "maximum urinary concentration" (mOsm/Kg. of water). Urinary osmolality under these conditions does not normally rise quite to the heights achieved after preliminary dehydration (6, 7).

Maximum urinary concentration in 13 thyrotoxic subjects varied from 640 mOsm/K to 954 mOsm/K, averaging 818 mOsm/K. This compares favorably with the renal concentrating ability of subjects without cardiovascular or renal disease tested in a similar fashion by Boyarsky and Smith (8), who found an average maximum urinary concentration of 789 mOsm /K (range, 614 to 975). The average urinary osmalality after administration of vasopressin in 10 apparently healthy medical students tested in this laboratory was 905 mOsm/K (range, 680 to 1180).

The administration of desiccated thyroid has been reported to produce diuresis in men (9), dogs (10), rats (11), and rabbits (12). The polyuria of diabetes insipidus can be ameliorated by thyroidectomy (13, 14) and is exacerbated by the administration of desiccated thyroid (15). The effects of thyroxine on urine flow are consistent with the belief that its action results from increasing the quantity of solute requiring excretion, although it has been suggested that hyperthyroidism may accelerate the destruction of antidiuretic hormone or decrease the sensitivity of the renal tubules to the action of vasopressin (5). Because hyperthyroidism is commonly associated with an increased urinary excretion of calcium and occasionally with an elevated concentration of calcium in the serum, it is possible that impairment of renal concentrating ability in some patients with thyrotoxicosis may be ascribable to nephrocalcinosis and/or hypercalcemia (16). The present results suggest that, despite the increased urinary output of solutes presumably resulting from increased appetite, most patients with hyperthyroidism are able to concentrate the urine to normal levels in response to large amounts of exogenous vasopressin.

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COMPLICATIONS DURING ADMINISTRATION OF METHYLESTRENOLONE

TO THE EDITOR:

Peters and co-workers (*J. Clin. Endocrinol. & Metab.* **18:** 114, 1958) reported 2 cases of jaundice seen during prolonged treatment with large doses of methylestrenolone. We have also observed jaundice combined

with 2 other complications which, in the interest of 19-*nor*-steroid therapy, are briefly described in this communication.

Patient #1 was a O-para IV-gravida who was treated with 15 mg. of methylestrenolone daily, starting at the end of the ninth week of pregnancy. Within six weeks there was marked water retention. The face, hands and feet became distinctly swollen and there were also manifestations suggestive of an androgenic effect, namely, a lowering of the pitch of the voice, pronounced seborrhea of the face and scalp, and extensive acne covering the face and the front and back of the chest. Dehydration was attained by rest and a saltless diet. The patient lost 4 Kg. within a week.

Jaundice slowly developed after two months. The serum bilirubin concentration increased to 19 mg. per 100 ml. A thymol turbidity test yielded negative results, zinc turbidity amounted to 0.5 unit, and the alkaline phosphatase level was 14 King-Armstrong units per 100 ml. Paper electrophoresis of the serum proteins showed the following values: total protein 7.2 Gm. per 100 ml.; albumin 44 per cent; alpha-globulins 16 per cent, beta-globulins 19 per cent, and gamma-globulins 21 per cent. Fiessinger's test revealed a disturbance in galactose clearance. The prothrombin time was prolonged (26/13). On the basis of these findings, the condition was considered to be one of intrahepatic cholangiolytic obstructive jaundice without any serious disturbances in hepatic function. Only the prolonged prothrombin time, which was not brought to normal by the administration of vitamin K, indicated disturbed function of the liver. Treatment was continued with 5 mg. of methylestrenolone daily, under strict control. Jaundice remained unchanged.

The patient gave premature birth to a male child weighing 1400 grams, after a 31-week pregnancy. The infant showed no anomalies at birth, but died on the fourth day post partum due to immaturity of the lungs. Autopsy revealed no abnormalities apart from the pulmonary condition. After birth, when methylestrenolone therapy was stopped, jaundice disappeared. After three months, the results of all liver function tests were normal. The voice, however, remained low in pitch.

Thus, in addition to jaundice, fluid retention and an androgenic effect were observed as complications of methylestrenolone therapy.

Patient #2 was treated with 15 mg. of methylestrenolone daily for habitual abortion. Pronounced retention of fluid occurred after four weeks. There was also virilization (lowered pitch of the voice, growth of hair on the chin and upper lip, and acne). The patient had a miscarriage at 4½ months of pregnancy.

These complications, especially the androgenic effect, should be regarded as warnings against *prolonged* treatment with *large doses* of methylestrenolone during pregnancy.

It should be emphasized that no side-effects were observed following administration of dosages up to 10 mg. daily over a limited period of time.

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The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Frank Engel, Duke Hospital, Durham, North Carolina, not later than *February 1, 1959. It is imperative that the abstracts be informative and complete with results and conclusions*—not a statement that those will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately two hundred abstracts submitted each year and the compiling of the program from these involves a tremendous amount of time and effort on the part of the Program Committee. The Council requests that the authors adhere strictly to the following rules when submitting abstracts, otherwise they cannot be considered:

1. *It is assumed that abstracts submitted for this program have not and will not be submitted elsewhere.*
2. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the

text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of the nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
5. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society.

Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15, 1958.*

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15, 1958.*

The American Goiter Association

THE 1959 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, on *April 30, May 1 and 2, 1959.*

THE VAN METER PRIZE AWARD FOR 1959

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in the Drake Hotel, Chicago, Illinois, April 30, May 1 and 2, 1959.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, not later than *January 15, 1959.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend.

JOHN C. MCCLINTOCK, M.D.
Secretary

20TH CONGRESS OF THE POLISH SOCIETY OF INTERNAL MEDICINE

The 20th Congress of the Polish Society for Internal Medicine will be held in Gdańsk, Poland, on *September 10–12, 1959*. The main problems to be discussed are 1) atherosclerosis, and 2) treatment with adrenocortical steroids. Applications to take part in the Congress are to be sent to the Chairman of the Executive Committee, 20th Congress of the Polish Society of Internal Medicine—Prof. Dr. Marian Górski, 1st Clinic of Internal Diseases ul. Orzeszkowej, Gdańsk-Wrzeszcz, Poland.

BOOK REGISTER

A Classified Bibliography of Gerontology and Geriatrics. Supplement One, 1949–1955. By NATHAN W. SHOCK, Chief, Gerontology Branch, National Heart Institute, National Institutes of Health, Bethesda, and the Baltimore City Hospitals, Baltimore, Maryland. Published under a grant from The Forest Park Foundation, Peoria, Illinois. The original Bibliography covered the period 1900 to 1948 and since then almost as many articles on the subject have been published; these are covered in the present Supplement, and attest to the steep rise in interest in the problems of aging. The references are classified according to: Gerontology, general orientation; Biology of Aging; Organ Systems; Geriatrics; Psychological Processes; Social and Economic Aspects; Popular Articles; Journals cited; Index of Authors; and Index of Subjects. This bibliography should be welcomed by all interested in gerontology. 1957, 553 pages. Stanford University Press, Stanford, California. Price $15.00.

Clinical Endocrinology. Second edition. By KARL E. PASCHKIS, M.D., Associate Professor of Medicine, Associate Professor of Physiology, Director of the Division of Endocrine and Cancer Research, Jefferson Medical College, ABRAHAM E. RAKOFF, M.D., Clinical Professor of Obstetric and Gynecologic Endocrinology, Jefferson Medical College, and ABRAHAM CANTAROW, M.D., Professor of Biochemistry, Jefferson Medical College, Philadelphia, Pennsylvania. The sections cover: Anterior Pituitary, Posterior Pituitary, Thyroid, Adrenal Cortex, Adrenal Medulla, Ovaries, Placenta, Testes, Pancreas, Parathyroids, Obesity, and Methods and Materials. Each gland is dealt with from the viewpoint of 1) anatomy and physiology, 2) conditions of hyperfunction, and 3) conditions of hypofunction. There are special sections on obesity, diabetes, infertility, puberty, adolescence, menstrual disturbances, pregnancy and the menopause. New material is presented on developments in the field of thyroid pathology and therapy (triiodothyronine, and hypothyroidism due to abnormal hormone synthesis) and in adrenal pathology and therapy (aldosteronism, virilizing adrenal hyperplasia, and the use of synthetic modified adrenocortical steroids), on the use of long-acting progesterone derivatives, and on the classification and diagnosis of testicular failure, among other subjects; 953 pages; 274 illustrations (6 in full color), 1958, Paul B. Hoeber, Inc., New York, N. Y. Price $18.00.

Report of the Medical Research Council for the Year 1956–1957. Presented to Parliament by the Lord President of the Council by Command of Her Majesty, June, 1958. There are short articles on some aspects of medical research (the architecture of a protein molecule, influenza in 1957, the mechanism of fever, female sex hormones and their quantitative estimation, and experimental leukemia, to mention a few) followed by a summary of the work carried out, and lists of scholarships and committee members; 280 pages. London, Her Majesty's Stationery Office. Price, 13 s 0 d net.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Administrative Medicine. Fifth Conference, October 29–31, 1956, Princeton, N. J. Edited by GEORGE S. STEVENSON, M.D., National and International Consultant, National Association for Mental Health, New York, N. Y. The programs covered are: Mental Health Elements in Administration of General Health

Programs; The Administrative Role in the Management of a Mental Hospital; and Coordination of Local Resources—Techniques and Devices for Communication and Cross-Fertilization; 197 pages; 1958. Price $3.75.

Physiology of Prematurity. Second Conference, March 25–27, 1957, Princeton, N. J. Edited by JONATHAN T. LANMAN, M.D., Department of Pediatrics, New York University—Bellevue Medical Center, New York, N. Y. There are 2 main sections in this book: 1) Aerobic and Anaerobic Metabolism in the Fetus and the Newborn, and 2) Breathing and Its Control in Premature Infants; 160 pages; 62 figures (1 in color); 23 tables; 1958. Price $3.75.